



# *Purification and polymerisation of microbial D-lactic acid from DDGS hydrolysates fermentation*

Article

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21 **Abstract**

22 A multi-step process was developed for microbial D-lactic acid purification, followed  
23 by poly-D-lactic acid (PDLA) synthesis via azeotropic polycondensation process. Several  
24 anion exchange resins were screened for their binding capacity using model lactic acid  
25 solutions. Amberlite® IRA67 (weak base anion exchange resin) showed the highest lactic acid  
26 adsorption, with maximum adsorption capacity,  $q_{max}$ , of 136.11 mg lactic acid / g of resin, and  
27 was further selected to purify D-lactic acid from DDGS hydrolysates through a three-step  
28 process; (1) treatment with 7% w/v activated carbon, (2) acidification of fermentation broth  
29 (Amberlite® IRA120) and (3) adsorption of lactic acid by anion exchange (Amberlite® IRA67).  
30 At the end of the purification process, 80.4% (w/w) D-lactic acid was recovered with 91.8%  
31 (w/w) purity, indicating the effectiveness of the developed downstream process. Furthermore,  
32 a clear yellowish solid polymer with a molecular weight of 3010 Da was obtained, suitable for  
33 applications in biomedical and agricultural sectors.

34

35 **Keywords:** purification, activated carbon, ion exchange resin, poly-D-lactic acid, azeotropic  
36 polycondensation, DDGS

37

38

## 39 1. Introduction

40 The world demand for lactic acid has increased significantly over the past few years, as  
41 the application of polylactic acid (PLA), offers distinctive advantages over petroleum-based  
42 polymers. PLA is a biodegradable plastic that can be used in biomedical and pharmaceutical  
43 industries as surgical suture, tissue engineering scaffolds or as drug delivery tool [1]. In the  
44 early stages of commercialisation, PLA was only produced for biomedical device applications  
45 due to its high cost [2]. Nowadays, the application of PLA has expanded to the electric and  
46 electronic industries for the production of casings and circuit boards, as well as in food industry  
47 for the production of food packaging and cutlery materials [3, 4]. Currently, the main producers  
48 of PLA are NatureWorks® LLC under the trade name Ingeo™, Cereplast, Inc. (United States),  
49 Corbion Purac (Netherlands), Toray Industries (Japan) and Zhejiang Hisun Biomaterial Co.,  
50 Ltd (China) [2]. Report by IHS Markit [5], predicted that PLA will be the leading application  
51 of lactic acid by 2020. PLA offers advantages over conventional petrochemical-derived plastics  
52 being biodegradable and compostable [2], and reduces the reliance on fossils fuel for the  
53 production of plastics [6]. Moreover, PLA production has a lower environmental impact  
54 compared to conventional petroleum derived polymers, as the carbon emissions and the energy  
55 consumption are reduced by 15 to 60% and 25 to 55%, respectively [7]. The low toxicity of  
56 PLA, along with its positive environmental characteristics, has rendered it an ideal material for  
57 application in various fields, including the food, biomedical and agricultural sector [6].

58 The production of PLA from agricultural **residues** represents a promising route for  
59 production, as such biomass is available at low cost, is accessible throughout the year and does  
60 not compete with food crops. For example, corn stover [8] and rice bran [9] have been identified  
61 as potential fermentation substrates for D-lactic acid production. However, the fermentation  
62 broths derived from renewable sources contain a mixture of compounds, including a variety of

63 sugars and proteins, polyphenols and organic acids, and thus require an effective downstream  
64 processing for the successful recovery of the targeted compound before being used as monomer  
65 for polymer synthesis [10].

66 Several downstream processing techniques such as ion exchange chromatography,  
67 precipitation, solvent extraction, distillation, nanofiltration, membrane extraction and  
68 electrodialysis have been investigated for the recovery and purification of lactic acid from  
69 fermentation broths [11-15]. Among these, adsorption by ion exchange offers a distinct  
70 advantage as it is a simple and relatively cheap process that offers product specificity, which  
71 leads to high purification yields [14, 16]. In organic acid separation, anion exchange resins are  
72 widely used. However, no specific conclusions on the optimum conditions for lactic acid  
73 binding have been drawn so far for anion exchange resins. For example, some researchers  
74 reported that a solution pH above the pKa of lactic acid (pKa lactic acid, 3.86) give the highest  
75 binding of lactic acid to Amberlite® IRA67 [17], Amberlite® IRA96 [16] and Amberlite®  
76 IRA92 [18]. On the other hand, other studies have found that a pH below the pKa value give  
77 the highest adsorption of lactic acid and other carboxylic acids to Amberlite® IRA67 [19],  
78 Amberlite® IRA35 [20, 21] and Lewatit S3428 resins [22]. To promote lactic acid binding on  
79 an anionic resin below the pKa value, the fermentation broth was acidified by treatment with  
80 strong acid or by passing the broth through a strong acidic cation exchange resin, i.e. Duolite  
81 C-464, to convert lactate salt to lactic acid [21].

82 Ring opening polymerisation and direct polycondensation are the most common  
83 methods used to synthesise PLA from lactic acid. In ring opening polymerisation, PLA is  
84 polymerised through a cyclic lactide intermediate. Companies such as NatureWorks® LLC  
85 (United States) and Corbion N.V. (Netherlands), produce PLA through this route [4, 23, 24].  
86 This protocol is of interest as it produces high molecular weight PLA. However, the procedure  
87 is complicated and time consuming because it involves several polymerisation steps and

88 requires high purity of the lactide monomer prior to PLA synthesis [25]. On the other hand,  
89 direct polycondensation offers significant advantages as the polymerisation process is simpler  
90 and easier in this case. In direct polycondensation, only one step for polymer synthesis is  
91 involved, during which the lactic acid solution is heated at 130 – 140 °C. Through this process,  
92 normally low molecular weight PLA (< 5000 Da) is produced with relatively weak mechanical  
93 properties; this is due to difficulties in removing the water from the reaction mixture as the  
94 polymerisation process progresses [26]. However, Ajioka, Enomoto, Suzuki and Yamaguchi  
95 [27] successfully produced high molecular weight PLA (> 300000 Da) using a single step  
96 synthesis using organic solvent with a catalyst (tin, Sn, powder) in azeotropic condition.  
97 Azeotropic polycondensation involved refluxing of the solvent under reduced pressure to  
98 remove the condensation water that was generated during polymer synthesis. This method had  
99 been patented and used by Mitsui Toatsu Chemicals (Japan) to synthesise PLA under the  
100 commercial name LACEA [6, 23].

101         The aim of the study was to develop a multi-step process for the purification of D-lactic  
102 acid from a fermentation broth based on dried distiller's grains with solubles (DDGS)  
103 hydrolysate [28]. Subsequently, the purified D-lactic acid was used as monomer for PDLA  
104 synthesis by employing an azeotropic polycondensation approach. As the purification of D-  
105 lactic acid from fermentation broth hydrolysates and its polymerisation process is rarely been  
106 reported, this study provides novel information on D-lactic acid separation, employing a  
107 multiple purification step followed by single step polymerisation process.

108

## 109 2. Materials and Methods

### 110 2.1 Materials

111 Dried Distillers Grains with Solubles (DDGS) was supplied from a bioethanol plant  
112 (Vivergo, Yorkshire, UK) and was alkaline pretreated as described by Zaini et al. [28]. Alkaline  
113 treated DDGS consisted of 52.6 g glucose, 25.0 g xylose, 10.3 g arabinose, and 0.04 g protein  
114 per 100 g of dried material. The resins (Amberlite<sup>®</sup> IRA67, Diaion<sup>®</sup> WA30, Amberlite<sup>®</sup>  
115 IRA400, Dowex<sup>®</sup> Marathon<sup>™</sup> MSA and Amberlite<sup>®</sup> IRA120) and activated carbon used in  
116 this study were purchased from Sigma-Aldrich (US).

117

### 118 2.2 D-lactic acid production

119 D-lactic acid was produced by *L. coryniformis* subsp. *torquens* (DSM 20004) using a  
120 Simultaneous Saccharification and Fermentation (SSF) process of alkaline treated DDGS in a  
121 2 L stirred tank bioreactor (Biostat B, Sartorius, Germany) [28]. The process was initiated by  
122 the simultaneous addition of Accellerase<sup>®</sup> 1500 (1 ml enzyme : 0.33 g cellulose) and *L.*  
123 *coryniformis* inoculum (starting OD of ~0.05) and was carried out for 30 hours (1.5 L  
124 fermentation medium). The SSF process was carried out at 37°C with an initial agitation speed  
125 of 250 rpm. The pH of fermentation medium was maintained at 5 with aseptic additions 5M  
126 NaOH and HCl through a peristaltic pump. The minimum dissolved oxygen (DO) level was  
127 kept at 20% by controlling automatically the stirrer speed. The culture containing the enzyme  
128 was inactivated by heat treatment at 95 °C for 10 minutes, followed by centrifugation at 17,105  
129 *x g* for 20 minutes (4 °C). Supernatants containing D-lactic acid solutions were collected and  
130 kept at -20 °C for purification.

131



## 132 2.3 Resin preparation

133 Weak anion exchange resins (Amberlite® IRA67 and Diaion® WA30) and strong anion  
134 exchange resins (Amberlite® IRA400 and Dowex® Marathon™ MSA) were selected for this  
135 study. Before utilisation, the resins in free base form, were first converted to Cl<sup>-</sup> form as  
136 described by Moldes et al. [29]. Resins that were purchased in Cl<sup>-</sup> form were only washed with  
137 distilled water [11, 30]. For the acidification of the fermentation broth, a cation exchange resin,  
138 Amberlite® IRA120, a strongly acidic resin in H<sup>+</sup> form, was used. The resin was washed with  
139 distilled water three times to remove any contaminants. All resins were then oven dried at 50  
140 °C overnight and stored at room temperature in closed containers before use. The properties of  
141 the ion exchange resins that were used in this study are presented in Table 1.

142

## 143 2.4 Screening and optimisation of anion exchange resins binding and recovery

144 For the screening experiments of the anion exchange resins, model lactic acid solutions  
145 were prepared using commercial lactic acid (85%, Food Chemical Codex, FCC, Sigma-  
146 Aldrich).

147

### 148 2.4.1 Effect of pH on lactic acid binding

149 The effect of pH on lactic acid binding was determined through batch experiments  
150 according to Bishai et al. [16] with slight modifications. 1 g of dried resin (Amberlite® IRA67,  
151 Diaion® WA30, Amberlite® IRA400 or Dowex® Marathon™ MSA) was mixed with 5 ml of  
152 lactic acid (50 g/l) at different initial pH conditions (2, 3, 4, 5, 6, 7 and 8) at 25 °C. The pH of  
153 the solutions was adjusted with 5 M NaOH. The mixtures were shaken at 200 rpm for 8 hours.

154 The liquid fractions from each mixture were collected by filtration and analysed for lactic acid  
155 concentration by HPLC.

156

#### 157 2.4.2 Effect of temperature on lactic acid binding

158 In order to investigate the effect of temperature on lactic acid binding, 1 g of each dried  
159 resin (Amberlite® IRA67, Diaion® WA30, Amberlite® IRA400 or Dowex® Marathon™ MSA)  
160 was mixed with 5 ml lactic acid (50 g/l), prepared at the optimum pH (obtained in Section  
161 2.4.1) and incubated at temperatures of 25, 30, 40, 50 and 60 °C at 200 rpm. The mixtures were  
162 shaken at 200 rpm for 8 hours. The liquid fractions from each mixture were collected by  
163 filtration and analysed for lactic acid concentration by HPLC.

164 The binding capacity,  $q$ , and adsorption efficiency,  $E$ , of the resin at different pH and  
165 temperatures were calculated as follows, as described by Pradhan et al. [11]:

$$q = \frac{(C_i - C_f) * V}{R} \quad (1)$$

$$E = \frac{(C_i - C_f)}{C_i} * 100 \quad (2)$$

166 where,  $q$  is the amount of lactic acid adsorbed to the resin (mg/g),  $E$  is the efficiency of lactic  
167 acid binding (%),  $C_i$  is the initial concentration of lactic acid (g/l),  $C_f$  is the concentration of  
168 lactic acid after being mixed with the resin (g/l),  $V$  is the volume of lactic acid solution (l) and  
169  $R$  is the weight of the resin (g).

170

#### 171 2.4.3 Adsorption capacity of lactic acid by resins

172 Amberlite® IRA67 and Diaion® WA30 were selected to carry out adsorption isotherm  
173 analysis in batch operation as described by Bernardo et al. [31] and John et al. [17], with slight  
174 modifications. 1 g of each resin was mixed with 5 ml of lactic acid at various concentrations

175 (4 - 650 mg lactic acid per 5 ml). The initial pH of the lactic acid solutions was set taking into  
176 account their optimum binding pH (obtained in Section 2.4.1) for the respective resin. The  
177 reaction took place at optimum temperature (obtained in Section 2.4.2) at 200 rpm for 8 hours.  
178 Liquid samples from each mixture were filtered and collected for further analysis.

179 Three different nonlinear models, the Langmuir, Freundlich and Langmuir - Freundlich  
180 models, were then fitted to the data using Origin Pro 8.0 software (OriginLab, USA) [32] using  
181 the following equations:

Langmuir model 
$$q = \frac{q_{max} * K * C}{K * C + 1} \quad (3)$$

Freundlich model 
$$q = K_f * C^{\frac{1}{n}} \quad (4)$$

Langmuir – Freundlich model 
$$\frac{q}{q_{max}} = \frac{K_{LF} * C^{nLF}}{1 + K_{LF} * C^{nLF}} \quad (5)$$

182 where,  $q$  is the amount of lactic acid adsorbed to the resin (mg/g),  $q_{max}$  is the maximum amount  
183 of lactic acid adsorbed to the resin (mg/g),  $K$  is the Langmuir adsorption constant,  $K_f$  is the  
184 Freundlich adsorption constant,  $K_{LF}$  is the affinity constant for adsorption,  $C$  is the amount of  
185 lactic acid (mg),  $n$  is the Freundlich adsorption constant and  $nLF$  is the Langmuir-Freundlich  
186 coefficient.

187

#### 188 2.4.4 Effect of ionic strength of eluent (HCl) on lactic acid recovery

189 The resin that exhibited the highest binding capacity,  $q$ , (Amberlite® IRA67) was  
190 selected for further recovery experiments using HCl with different ionic strengths as eluent. 4  
191 g of Amberlite® IRA67 were packed into a 100 mm (length) column (Fisher Scientific,  
192 Leicester, UK) and saturated with 20 ml of 30 g/l lactic acid solution. The resin was then  
193 washed with distilled water to remove any unbound lactic acid. Different concentrations of HCl

194 were used as eluent (0.05, 0.1, 0.5 and 1.0 M), which were passed down the packed column by  
195 gravity and fractions of effluents were collected (2 ml per fraction) at regular time intervals.  
196 All fractions were analysed for lactic acid concentration.

197

## 198 2.5 Purification of D-lactic acid from fermentation broth

### 199 2.5.1 Colour removal by activated carbon

200 The effect of activated carbon on the removal of the colour from the fermentation broth  
201 was determined. Powdered activated carbon was mixed with 5 ml of clarified fermentation  
202 broth at different loading concentrations (0, 1, 5, 7 and 10%, w/v), for 1.5 hours at 150 (25 °C).  
203 The mixture was then separated by centrifugation at  $17,105 \times g$  for 10 minutes (4 °C). The pellet  
204 was washed twice with distilled water and the supernatants collected for sugar and lactic acid  
205 analysis.

206

### 207 2.5.2 Acidification of fermentation broth by cation exchange resin

208 After treatment with activated carbon, the fermentation broth was subjected to  
209 Amberlite® IRA 120, H<sup>+</sup> resin aiming to convert sodium lactate into lactic acid [33]. 10 g of  
210 dried resin were packed into a 30 cm length Econo-glass column (i.d. 1 cm) which was first  
211 filled with distilled water and the height of the resin was fixed with a flow adaptor (i.d. 1 cm)  
212 (Biorad, California, US). Distilled water was allowed to pass through the column until the pH  
213 of effluent was around 6.5. Then, a constant flow of 3 ml/min using a peristaltic pump was  
214 applied. The fermentation broth containing sodium lactate with a pH around 5.5 was then  
215 pumped into the column at the same flow rate. When the pH of the effluent started to increase,  
216 the resin was considered saturated. Effluent fractions (4 ml each) were collected for lactic acid

217 and sugar analysis. Fractions containing D-lactic acid were pooled for subsequent purification.  
218 The resin was washed with distilled water to remove the remaining solution in interstitial  
219 spaces, regenerated with 1 M HCl and thoroughly rinsed with distilled water, before a new  
220 cycle [16].

221

### 222 2.5.3 Adsorption by anion exchange resin

223 Pooled supernatant fractions containing D-lactic acid were passed through an anion  
224 exchange resin, (Amberlite<sup>®</sup> IRA67) in fixed-bed column operation. 25 g of dried resin were  
225 packed into a 30 cm length Econo-glass column (i.d. 2.5 cm) which was first filled with distilled  
226 water and the upper side of resin was fixed with a flow adaptor (i.d. 2.5 cm). The system was  
227 washed with distilled water until the pH of the effluent was around 6.5. Then, the acidified  
228 broth obtained from Amberlite<sup>®</sup> IRA 120, H<sup>+</sup> resin was pumped into the column at 3 ml/min.  
229 D=lactic acid was recovered with 0.5 M HCl. Fractions of effluents at each stage were collected  
230 (4 ml each) for lactic acid and sugar analysis.

231

### 232 2.6 Azeotropic polycondensation process of PDLA

233 Polycondensation of PDLA was conducted as described by Ajioka et al. [27] with minor  
234 modifications. 2 g of D-lactic acid were mixed with 40 ml toluene in 100 ml reaction flask,  
235 equipped with a Dean-stark apparatus and a magnetic stirrer. In the first step of the  
236 polycondensation process, the mixture was azeotropically dehydrated at 110 °C for 3 hours to  
237 remove the free water. After removing the condensed water that was trapped in the Dean-stark  
238 apparatus, the tube was packed with molecular sieve (4 Å) and calcium chloride in layers to  
239 remove small amounts of water dissolved in the organic solvent that was produced during PLA  
240 synthesis. 0.2 g tin (II)-2-ethylhexanoate (stannous octoate) was added to the reaction mixture

241 and then returned to reflux at 140 °C for another 80 hours. The polymer produced was then  
242 recovered by filtration and vacuum dried using Büchner funnel apparatus, followed by freeze  
243 drying (VisTis Sentry 2.0, Warminster, PA).

244

## 245 2.6 Analytical methods

246 The nitrogen content was determined using the Free Amino Nitrogen (FAN) method as  
247 described by Lie [34] with some modifications. 0.5 ml of diluted sample was mixed with 0.25  
248 ml of colour reagent (49.71 g of Na<sub>2</sub>HPO<sub>4</sub>·2H<sub>2</sub>O, 5 g of ninhydrin, 3 g of fructose and ~ 40 g  
249 of KH<sub>2</sub>PO<sub>4</sub> dissolved in 1 l of distilled water; pH 6.6 – 6.8) in 2 ml Eppendorf tube. The mixture  
250 was heated at 100 °C in a thermal block (Grant, Cambridge) for exactly 16 minutes and  
251 immediately cooled in an ice bath. 1.5 ml of dilution reagent (2 g potassium iodate, KIO<sub>3</sub>, in  
252 616 ml distilled water and 384 ml 96% ethanol) was added and the free amino nitrogen content  
253 was measured at 570 nm. A calibration curve was constructed using glycine at different  
254 concentrations (0.25 – 2 mg/l) as standard.

255 Sugar and lactic acid concentrations were analysed by high performance liquid  
256 chromatography (HPLC) in an Agilent Infinity 1260 system (Agilent Technologies, USA)  
257 equipped with an Aminex HPX-87H column (Bio-rad, Hercules, CA) at a 0.6 ml/min flow rate  
258 with 5 mM H<sub>2</sub>SO<sub>4</sub> as mobile phase. The temperature of the column was set at 65 °C and sugars  
259 and lactic acid were detected using a refractive index detector. The D-lactic acid recovery and  
260 purity were calculated using the following equations [16]:

$$261 \text{ Recovery (\%)} = \frac{\text{mg LA in each purification stage}}{\text{mg LA in fermentation broth}} * 100 \quad (6)$$

$$262 \text{ Purity (\%)} = \frac{\text{HPLC peak area of LA in each purification stage}}{\text{Total HPLC peak areas in each purification stage}} * 100 \quad (7)$$

261 The molecular weight and poly dispersity index (PDI) of PDLA was determined by gel  
262 permeation chromatography (GPC), using an Agilent 1100 Series chromatography system that  
263 was equipped with a refractive index RID 1200 detector (35 °C). The flow rate was set at 1.0  
264 ml/min and the molecular size was determined using a PL gel 5 $\mu$ M mixed-D column (300 x  
265 7.5 mm) and a PL gel 5 $\mu$ M guard column (50 x 7.5 mm). Chloroform was used as the eluent.  
266 The PDLA obtained was first dissolved in chloroform prior to analysis. The sample was  
267 analysed at room temperature using 20  $\mu$ l injection volume. A calibration curve was generated  
268 using polystyrene standards with molecular weights ranging from 580 to 483,400 Da.

269

### 270 3. Results and Discussion

#### 271 3.1 Selection of anion exchange resin

272 When considering ion exchange chromatography, the efficiency of the product  
273 adsorption by the resin determines the success of the purification process. Therefore, factors  
274 such as pH, temperature and lactic acid concentration were initially investigated in this study  
275 in order to select the most appropriate anion exchange resin. The effect of the ionic strength of  
276 the eluent (HCl solution) on product recovery was also investigated. Before use, the resins that  
277 existed in free base were converted to Cl<sup>-</sup> form. The Cl<sup>-</sup> form was selected as previous studies  
278 have shown that in this form the resins exhibit the highest adsorption capacity for lactic acid  
279 [17, 31]. Moreover, the simultaneous lactic acid recovery and resin regeneration for subsequent  
280 adsorption cycles by HCl, reduces the number of steps involved in the purification process  
281 [29]. Four different resins, categorised into weak base anion exchange (Amberlite<sup>®</sup> IRA67 and  
282 Diaion<sup>®</sup> WA30) and strong base anion exchange (Amberlite<sup>®</sup> IRA400 and Dowex<sup>®</sup>

283 Marathon<sup>TM</sup> MSA) were tested against different initial pH values, ranging from 2 to 8, as shown  
284 in Figure 6a.

285         Among the tested resins, the weak base anion exchange resins showed capability for  
286 lactic acid binding at low pH, with the highest adsorption being 65% with Amberlite<sup>®</sup> IRA67  
287 at pH 3, which corresponded to a maximum binding capacity ( $q_{max}$ ) of 155 mg lactic acid/g of  
288 resin. For strong base anion exchange resins, less than 22% of lactic acid was adsorbed by both  
289 Amberlite<sup>®</sup> IRA400 and Dowex<sup>®</sup> Marathon<sup>TM</sup> MSA, with the highest binding at pH 6 and pH  
290 4, respectively, suggesting that the pH did not influence the adsorption of lactic acid to the  
291 strong base anion exchange resins. On the other hand, the adsorption of weak base anion  
292 exchange resins was strongly influenced by the pH of the feed solution. In the case of  
293 Amberlite<sup>®</sup> IRA67 and Diaion<sup>®</sup> WA30, the best pH for lactic acid adsorption was below its  
294 pKa (3.86), where lactic acid exists in its undissociated form [21, 22, 35]. This can be  
295 associated with the charge of the tertiary amine (the functional group in both resins) which is  
296 cross-linked to the polymeric matrix (acrylic or styrene) in Amberlite<sup>®</sup> IRA67 and Diaion<sup>®</sup>  
297 WA30. It is assumed that the lone pair electron of the nitrogen atom in the tertiary amine is  
298 likely to hydrogen bond to lactic acid through the chloride ion [20, 36]. This mechanism is also  
299 supported by works from Yousuf et al. [35], Kislik [13] and Kulprathipanja and Oroskar [20],  
300 who reported that possible interactions between amine-based extractants and carboxylic acids  
301 are through hydrogen bonding, acid-base interaction, hydrophobic interaction, ion-ion pair  
302 formation or solvation.

303         Subsequently, the effect of temperature (25 to 60 °C) on lactic acid adsorption was  
304 investigated at pH values that were previously shown to give the highest lactic acid binding for  
305 each resin (Figure 6b). No differences were observed between the different temperatures, for  
306 each resin. As shown previously, the highest lactic acid adsorption (~73%) was exhibited by  
307 Amberlite<sup>®</sup> IRA67. According to Niazi and Brown [37], the effect of temperature on ion



308 exchange resins is mainly attributed to pKa changes of the targeted compound as a result of the  
309 temperature change. In the case of lactic acid, as the temperature increased from 25 to 30, 40  
310 and 50°C, the pKa value also increased to 3.896, 3.942 and 4.028, respectively [38]. Since  
311 lactic acid adsorption to weak base anion exchange resins occurs below its pKa value, the  
312 increase in temperature from 25 to 60 °C did not have any significant effect.

### 313 3.1.1 Adsorption isotherms

314 The two resins that demonstrated the highest binding capacity, Amberlite® IRA67 and  
315 Diaion® WA30, were further tested. The adsorption isotherms were generated at 25 °C, and are  
316 shown in

317 Figure 7a and 2b, respectively. Different kinetic models (Langmuir, Freundlich and Langmuir  
318 – Freundlich) were then used to fit the data, and the model parameters are presented in Table  
319 2.

320 The Langmuir and Langmuir – Freundlich models fitted better the data for both resins  
321 ( $R^2 > 0.9$ ), compared to the Freundlich model ( $R^2 = 0.80$ ). The Langmuir – Freundlich model  
322 showed better fit than the Langmuir model for both the Amberlite® IRA67 ( $R^2 = 0.94$ ) and the  
323 Diaion® WA30 resin ( $R^2 = 0.965$ ). In the Langmuir model, it is assumed that the adsorption of  
324 a given adsorbate occurs as a monolayer sorption onto the surface of a resin containing a finite  
325 number of identical binding sites. The adsorbent has uniform binding sites and the adsorbate  
326 will only bind to the binding site [32, 39]. In the Freundlich model, it is assumed that the  
327 binding of the adsorbate molecules onto the adsorbent is at infinite capacity. The adsorption is  
328 not uniform and can occur in a multilayer, with the binding site that has stronger bond energy  
329 being occupied first [39]. In the present study, the adsorption capacity of the resin was specific  
330 due to the strong interactions between lactic acid and the functional groups present in the Cl<sup>-</sup>  
331 form of the resin. As a result, the Freundlich model did not fit as well as the other models the

332 kinetic data. According to Sala et al. [32], the Langmuir-Freundlich model is the simple  
333 generalisation of both isotherms, modelling the adsorption cooperativity of the two different  
334 binding mechanisms. This level of cooperativity can be determined from the  $nLF$  value, where,  
335 when  $nLF > 1$ , a positive cooperativity is indicated. When  $0 < nLF < 1$ , a negative cooperativity  
336 in binding process is indicated, whereas when  $nFL$  value = 1, it is assumed that the adsorption  
337 is purely independent, and no interaction takes place between adsorbents. In this study, the  $nFL$   
338 values for both resins were  $> 1$ , with 1.96 for Amberlite® IRA67 and 1.83 for Diaion® WA30,  
339 indicating a positive cooperativity of the two binding mechanisms.

340 The maximum binding capacity of a resin ( $q_{max}$ ) can also be predicted from the  
341 Langmuir and Langmuir–Freundlich models, however it cannot be obtained from the  
342 Freundlich model as this model assumes that lactic acid binding to the resin is unlimited. The  
343 highest  $q_{max}$  values for Amberlite® IRA67 were 162.09 and 136.11 mg/g resin based on the  
344 Langmuir and Langmuir–Freundlich models, respectively. This value, however, was lower  
345 than the  $q_{max}$  value reported by Garrett et al. [40], i.e. 203 mg/g of resin, as predicted by the  
346 Langmuir model.

347

### 348 3.1.2 Effect of HCl strength on the recovery of lactic acid

349 The strength of the eluent on the recovery of lactic acid plays a key role for ensuring  
350 that all of the lactic acid that is bound to the resin is detached. In this study, HCl was used as  
351 an eluent to recover lactic acid from Amberlite® IRA67, which was deemed from the previous  
352 work to be the most suitable resin. Figure 8 depicts the elution profiles of lactic acid at different  
353 HCl concentrations. At 0.05 M and 0.1 M HCl, only 5.1 and 22.1% lactic acid was recovered  
354 from Amberlite® IRA67, respectively. However, at 0.5 and 1.0 M, ~ 96% and 100% recovery  
355 of lactic acid was achieved, respectively, indicating that the strength of the eluent plays critical

356 role for the detachment of lactic acid from the resin. Based on these results, 0.5 M HCl was  
357 selected to recover lactic acid in subsequent experiments.

358

## 359 3.2 Separation and purification of D-lactic acid from fermentation broth

### 360 3.2.1 Colour removal by activated carbon

361 After determining the conditions leading to maximum lactic acid adsorption and  
362 recovery using the Amberlite® IRA67 resin, the aim was to purify lactic acid from fermentation  
363 broths of *L. coryniformis* subsp. *torquens*, where DDGS hydrolysate was used as the  
364 fermentation medium. Fermentation broths usually contain besides the component of interest,  
365 residual sugars and proteins, as well as by-products of the fermentation process. In this  
366 particular case, fermented DDGS hydrolysate contained residual sugars (xylotriose, xylobiose,  
367 xylose, arabinose), organic nitrogen in the form of proteins, peptides or amino acids,  
368 polyphenols and acetic acid, all of which can contributed into the dark brown colour of the  
369 fermentation broth [41]. It is likely that a significant proportion of the dark brown colour of the  
370 medium is due to the dark colour of DDGS generated during the drum drying step in the DDGS  
371 production process. Therefore, prior to ion exchange purification, the fermented DDGS  
372 hydrolysate was initially subjected to activated carbon treatment. Figure 9 shows the effect of  
373 various activated carbon concentrations on the colour of the fermentation broth. A positive  
374 correlation can be observed as the activated carbon concentrations increased up to 5% (w/v)  
375 and the colour of the fermentation broths became notably lighter. For higher activated carbon  
376 concentrations (7 and 10%, w/v) no significant changes in colour were observed.

377 The effect of activated carbon concentration on the recovery of D-lactic acid, protein,  
378 oligosaccharides and monosaccharides is shown in Table 3. The recovery of D-lactic acid  
379 gradually decreased as the concentration of activated carbon increased. More specifically at 1,

380 5, 7 and 10% (w/v) of activated carbon, 95, 90, 88 and 85% lactic acid was recovered from the  
381 fermentation broth, respectively. A relatively small reduction in monosaccharides and proteins  
382 concentrations was overall noted (< 30% removal) when the fermentation broth was treated  
383 with activated carbon, even as high as 10% (w/v). Stone and Kozlov [42] reported that activated  
384 carbon can only adsorb low molecular weight proteins, in which is in line with protein removal  
385 data obtained in this study. On the other hand, oligosaccharides (xylotriose and xylobiose) were  
386 completely removed at 7% (w/v) activated carbon. According to Boon et al. [43], activated  
387 carbon has higher affinity for trisaccharides and disaccharides compared to monosaccharides  
388 with a capacity of 133 mg/g and 117 mg/g of activated carbon, respectively. Based on the  
389 above, 7% (w/v) activated carbon was selected as the best concentration to treat the  
390 fermentation broth to ensure a satisfactory recovery of lactic acid and at the same time the  
391 removal of oligosaccharides and partially of proteins and monosaccharides.

392

### 393 3.2.2 Acidification of fermentation broth by cation exchange chromatography

394 In lactic acid fermentations, the pH of the culture is normally controlled to prevent  
395 microbial growth inhibition and ensure an adequate growth of lactic acid bacteria [44]. NaOH  
396 is often selected as the neutralising agent because of its low cost and the fact that no gypsum  
397 is generated as a by-product which is the case when  $\text{Ca}(\text{OH})_2$  is used [45]. As a result, sodium  
398 lactate is formed in the fermentation broth. In order to recover lactic acid by anion exchange  
399 chromatography, the pH of the broth needs to be reduced to below pH 3 in order for lactic acid  
400 to be in its undissociated form rather than in the form of salt. To achieve this and taking into  
401 account that the pH of the fermentation broth used in this study was ~5.5, the fermentation  
402 broth was passed through the Amberlite<sup>®</sup> IRA120 ( $\text{H}^+$ ) cation exchange resin, in order to  
403 exchange the sodium ions and release lactate ions into the solution. Figure 10a depicts the  
404 compositional profile of the solution during passing through the resin.

405 In cation exchange chromatography, no eluent is needed as lactic acid does not bind to  
406 Amberlite IRA<sup>®</sup> 120 (H<sup>+</sup>). Amberlite IRA<sup>®</sup> 120 is a strong acidic cation exchange resin that  
407 contains sulfonic acid functional groups. It is assumed that the hydrogen ions (H<sup>+</sup>) that are  
408 attached to the resin's functional group will bind to sodium lactate and convert it to lactic acid  
409 in the solution. At the same time, the cation of the lactate salt (Na<sup>+</sup>) will be transferred to the  
410 cation exchange resin and transformed through the reaction:  $P-H^+ + Na^+La^- \rightarrow P-Na^+ + H^+La^-$ ,  
411 where P is the polymer matrix [46]. In the first 12 ml of the fractions collected, no organic  
412 acids and sugars were detected and the pH of the collected fraction was around 6.5, indicating  
413 that the fraction contained only water (already present in the column). The pH of the solution  
414 then dropped sharply and when it reached ~2.3, the presence of D-lactic acid and other  
415 compounds such as acetic acid, xylose and arabinose were detected. The pH of eluate in the  
416 collected fraction dropped further with time, to ~1.5, indicating that sodium lactate was  
417 successfully converted to undissociated lactic acid. Fractions that had pH below 3 were then  
418 pooled together yielding a fraction with a pH of 1.67.

419

### 420 3.2.3 Recovery of D-lactic acid by anion exchange chromatography resin

421 The undissociated form of D-lactic acid collected from Amberlite<sup>®</sup> IRA120 (H<sup>+</sup>) was  
422 then subjected to Amberlite<sup>®</sup> IRA67 (Cl<sup>-</sup>). According to Bishai et al. [16], Amberlite<sup>®</sup> IRA67  
423 has significant commercial potential as it is a robust resin where the amines present in the  
424 functional group do not easily detach from the polymer matrix, it is easy to regenerate, and  
425 provides higher recovery of lactic acid compared to other resins. The results from this study  
426 (Figure 10b) show that during the binding stage, no lactic acid was present in the collected  
427 fraction, while other compounds such as xylose and arabinose were detected. The pH of the  
428 effluent collected during the binding stage was around 5.5 to 6.0, indicating that the organic  
429 acids present in the fermentation broth (lactic acid and acetic acid) were adsorbed to the resin.

430 After washing the column with water, lactic acid was desorbed with 0.5 M HCl. The first acid  
431 to be eluted was acetic acid, followed by lactic acid. Fractions that contained only lactic acid  
432 were pooled together.

433 Table 4 presents the recovery and purity of D-lactic acid, as well as the percentage of  
434 total sugars and protein removal after each stage of the purification process. After the  
435 fermentation broth was treated with 7% (w/v) activated carbon, a ~96% recovery of D-lactic  
436 acid recovery was observed and a 9.6% increase in D-lactic acid purity, the latter due to the  
437 complete removal of oligosaccharides and of 55% of the proteins that were initially present in  
438 the fermentation broth. During the cation exchange stage, a ~93% recovery of D-lactic acid  
439 compared to the D-lactic acid concentration in the fermentation broth was observed, and  
440 although only 1% increase in D-lactic acid purity was detected, ~21% of total sugar and ~44%  
441 of proteins were successfully removed at this stage. Cation exchange did not increase the D-  
442 lactic acid purity as its main objective was to acidify the fermentation broth. During the anion  
443 exchange step a ~80.4% recovery of D-lactic acid was observed, while 74% of the sugars were  
444 removed, leading to a cumulative sugar removal of 100%. As a consequence, the purity of D-  
445 lactic acid in the eluent was significantly increased during this step, reaching 91.8%, with the  
446 remaining components consisting most likely of small amounts of organic molecules, e.g.  
447 proteins, acetic acid and other microbial metabolites. Optical purity values for D-lactic above  
448 92% acid are considered appropriate for the synthesis of PLA [44] and demonstrate that the  
449 proposed multi-step downstream process has significant potential for scaling up and  
450 commercialisation.

451

### 452 3.3 PDLA synthesis

453 The azeotropic dehydration polycondensation method was employed using toluene as  
454 solvent and tin (II)-2-ethylhexanoate (stannous octoate) as catalyst during polymerisation of D-  
455 lactic acid obtained from microbial fermentation of DDGS. Tin compounds and protonic acid  
456 have been found to be the best catalysts for the direct polycondensation of high molecular  
457 weight PLA [27]. However, stannous octoate is preferable as it is approved by U. S. Food and  
458 Drug Administration (FDA) in the list of Indirect Additives used in Food Contact Substances  
459 under the Code of Federal Regulation, Title 21 (Food and Drugs). In addition to that, the  
460 removal of water is crucial in the direct polycondensation process, as its presence could initiate  
461 transesterification reactions or chain terminating reactions of the PLA produced [26, 47].  
462 During azeotropic polycondensation, the water that was present in the feedstock (D-lactic acid)  
463 or generated during the polymerisation process was continuously distilled off from the reaction  
464 mixture through the Dean-stark trapped apparatus.

465 After 80 h of polymerisation, a solid yellowish crystal PDLA was produced with an  
466 average molecular weight of 3010 Da with a PDI of 4.1, categorised as low molecular weight  
467 PLA. In contrast, Kim and Woo [48] produced ten times higher molecular weight of PLLA  
468 (33000 Da) after 72 h reaction when the same polycondensation method was used. A similar  
469 finding was reported by Marques et al. [49], where 80,000 Da molecular weight of PLLA was  
470 produced after 70 h of polymer synthesis. The differences in the molecular weight produced in  
471 both cases might be due to the differences in the type of catalyst (tin (II) chloride dihydrate)  
472 and solvent (*m*-xylene with higher than toluene by 29°C boiling point) used during the  
473 polymerisation process in those cases. Moreover, other factors such as the purity of D-lactic  
474 acid, minute amounts of carboxylic acids like acetic acid, the presence of small amounts water  
475 during the polymerisation process and the reaction temperature might also have contributed to  
476 the generation of low molecular weight PDLA [12]. Table 5**Error! Reference source not**

477 **found.** compares the molecular weight of PLA produced from this and other studies, where  
478 various synthesis methods, monomers (D- or L- lactic acid) and catalysts were employed.

479 This is the first work demonstrating the synthesis of D-lactic acid polymer (PDLA)  
480 employing either a direct method or a one-step polymerisation (azeotropic polycondensation)  
481 process. **Pivsa-Art et al. [50]** synthesised PDLA via a two-step polycondensation process,  
482 which included a melt polymerisation step followed by solid state polymerisation, and  
483 produced PLA with a molecular weight of 33300 Da. A number of interesting applications for  
484 low molecular weight PLA have been proposed in various fields, especially in the biomedical  
485 [47] and agricultural fields [51]. In the biomedical field, low molecular weight PLA is used as  
486 particles for parental controlled drug release in the human body in the form of microspheres,  
487 microcapsules, pellets or tablets. Using this approach, drugs are fabricated in a polymeric  
488 device (PLA) and the release of the drug is regulated by either diffusion through the polymer  
489 barrier or erosion of the polymer matrix [52]. PLA is preferable compared to other polymers  
490 such as polyethylene and silicon rubber, as it does not require surgical retrieval, due to its  
491 natural degradation in the body. PLA is degraded by simple non-enzymatic hydrolysis (after  
492 exposure to moisture) to its monomer (lactic acid) , which can be metabolised by the human  
493 body [47]. Drug delivery using low molecular weight PLA offers advantages over high  
494 molecular weight PLA as it has a weak retarding effect. Thus, the risk of material accumulation  
495 in tissues is reduced as PLA is relatively fast degraded to lactic acid [53].

496 The same mode of application (controlled release) is also being used in the agricultural  
497 field, specifically the agrochemical sector, where PLA can serve as carrier for herbicides and  
498 pesticides that are released into the soil. The advantages of using low molecular weight PLA  
499 have been demonstrated by Zhao and Wilkins [51], where delayed release of pesticides was  
500 observed in the early stages of application, which makes it desirable for sensitive targets such  
501 as seed treatment. In their study, bromacil (pesticide) was incorporated into PLA in the form



502 of granules and films, and the delayed release of bromacil was achieved via degradation and  
503 erosion of PLA. Since the degraded monomer (lactic acid) is safe and widely distributed in  
504 nature, the environmental problems of polymer disposal can be avoided through this approach  
505 [6, 51].

506 In addition, Quynh et al. [54] successfully produced PLA with high thermostability  
507 when commercial PLLA polymer (10400 Da) was crosslinked with low molecular weight  
508 PDLA (9830 Da). The stereo complex formation of PLLA and PDLA was achieved via the  
509 melt polycondensation process and increased the melting temperature of PLA from 175.6 °C to  
510 218.1 °C. This finding has widespread the application of PLA in other areas such as in the  
511 production of computer casings, automotive components and heat resistant food packaging [2].

512

#### 513 **4. Conclusions**

514 Low molecular weight PDLA (3010 Da) was produced via single step azeotropic  
515 polycondensation process from purified D-lactic acid originated from DDGS. The developed  
516 downstream process reduced effectively the dark colour of fermentation broth, removed  
517 oligosaccharides and converted sodium lactate to undissociated lactic acid. At the end of the  
518 purification process, approximately 80.4% D-lactic acid was recovered with 91.8% purity. This  
519 study demonstrated that agricultural residues, such as DDGS, hold potential as starting  
520 materials for biopolymer production.

521

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526

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683 Figure Captions

684 Figure 1: Effect of (a) pH and (b) temperature on lactic acid binding by four anionic resins

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686 Figure 2: Adsorption isotherms of lactic acid to (a) Amberlite® IRA67 and (b) Diaion®

687 WA30 at 25°C; - - - Langmuir model, ..... Freundlich model, \_\_\_ Langmuir-Freundlich

688 model

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690 Figure 3: Elution profiles of lactic acid from the Amberlite® IRA67 resin (saturated with 20 ml

691 of 30 g/l lactic acid) at different concentrations of HCl at 25°C; (●) 0.05M, (▲) 0.1M HCl,

692 (■) 0.5M HCl and (✕) 1.0M HCl.

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694 Figure 4: Samples of fermentation broth treated with different concentrations of activated

695 carbon at 25 °C for 1.5 h

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697 Figure 5: Elution profile of fermentation broth on (a) cation exchange chromatography with

698 Amberlite IRA120 (H<sup>+</sup>) resin and (b) adsorption and elution profile of fermentation broth

699 during anion exchange chromatography with Amberlite® IRA67 (Cl<sup>-</sup>) resin

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706 Table 1: Properties of ion exchange resins (information provided by Sigma-Aldrich, US)

<b>Ion exchange type</b>	<b>Resin</b>	<b>Strength</b>	<b>Particle size (µm)</b>	<b>Matrix</b>	<b>Active functional group</b>	<b>pH</b>
Anion	Amberlite® IRA67	Weak basicity	500-750	Acrylic (gel)	Tertiary amine	0 - 7
Anion	Diaion® WA30	Weak basicity	680	Styrene-divinylbenzene (highly porous)	Tertiary amine	0 - 9
Anion	Amberlite® IRA400	Strong basicity	600-750	Styrene-divinylbenzene (gel)	Quaternary ammonium	0 - 14
Anion	Dowex® Marathon™ MSA	Strong basicity	640	Styrene-divinylbenzene (macroporous)	Quaternary ammonium	0 - 14
Cation	Amberlite® IRA120	Strong acidity	620-830	Styrene-divinylbenzene (gel)	Sulfonic acid	0 - 14

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719 Table 2: Langmuir, Freundlich and Langmuir – Freundlich isotherm parameters describing  
 720 the adsorption of lactic acid to Amberlite® IRA67 and Diaion® WA30

<b>Model</b>	<b>Parameter</b>	<b>Amberlite® IRA67</b>	<b>Diaion® WA30</b>
Langmuir	$q_{max}$ (mg/g)	162.09	102.47
	$K$	0.0096	0.019
	$R^2$	0.910	0.940
Freundlich	$K_f$	10.88	13.379
	$n$	2.475	3.200
	$R^2$	0.801	0.800
Langmuir-Freundlich	$q_{max}$ (mg/g)	136.11	91.51
	$K_{LF}$	0.0002	0.001
	$n_{LF}$	1.96	1.83
	$R^2$	0.940	0.965

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733 Table 3: Recovery of D-lactic acid, free amino nitrogen, oligosaccharides and  
 734 monosaccharides after treatment with activated carbon at different concentrations

Activated carbon (%, w/v)	*Recovery (%)					
	D-lactic acid	Free amino nitrogen	Oligosaccharides		Monosaccharides	
			Xylotriase	Xylobiose	Xylose	Arabinose
0	100 ± 0.0	100 ± 2.5	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0
1	95 ± 0.4	94.0 ± 0.2	77.4 ± 0.5	87.6 ± 0.0	90.4 ± 0.9	83.4 ± 1.6
5	90 ± 0.3	83.6 ± 1.3	0	42.8 ± 1.0	85.7 ± 0.6	79.9 ± 0.9
7	88 ± 3.4	83.0 ± 1.2	0	0	82.2 ± 3.0	76.7 ± 2.7
10	85 ± 1.9	80.2 ± 2.8	0	0	72.7 ± 1.7	70.0 ± 1.4

735 \*Initial concentration in the fermentation broth: D- lactic acid, 25.9 g/l; xylotriase, 1.0 g/l; xylobiose,  
 736 3.8 g/l; xylose, 4.8 g/l; arabinose, 0.5 g/l and free amino nitrogen, 152.8 mg/l

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743 Table 4: Recovery and purity of D-lactic acid from the fermentation broth during the different downstream processing stages

Purification stages	Volume (ml)	D-lactic Acid			Total Oligosaccharides		Total Monosaccharides		Free amino nitrogen	
		g/l	Recovery (%)	Purity* (%)	g/l	Cumulative removal (%)	g/l	Cumulative removal (%)	mg/l	Cumulative removal (%)
Fermentation broth	50	25.9	100	54.6	4.5	0	5.3	0	152.8	0
7% Activated carbon	92	13.5	95.9	64.2	0	100	2.7	4.5	37.8	55
Cation exchange chromatography (Amberlite® IRA120, H <sup>+</sup> )	132	9.1	92.5	65.2	0	100	1.7	26.2	0.6	99
Anion exchange chromatography (Amberlite® IRA67, Cl <sup>-</sup> )	29	36.0	80.4	91.8	0	100	0	100	0.4	99

744 \*The purity (%) of lactic acid in the eluate was determined according to Equation

745 Table 5: Comparisons of molecular weight of PLA produced from this and other studies,  
 746 using different synthesis methods, catalysts and monomers were.

Method	Lactic acid form	Catalyst	Polymer	Molecular weight (Da)	Reference
Azeotropic polycondensation	D	Tin (II)-2-ethylhexanoate, $\text{SnC}_{16}\text{H}_{30}\text{O}_4$	PDLA	3010	This study
	L	Tin (II) chloride dihydrate, $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$	PLLA	33000	[48]
	L	Tin (II) chloride dihydrate, $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$	PLLA	80000	[49]
Direct polycondensation	L	-	PLLA	90000	[55]
	L	Antimony trioxide, $\text{Sb}_2\text{O}_3$	PLLA	67000	[56]
Melt polymerisation	DL	Tin (II) chloride, $\text{SnCl}_2$	PDLLA	4100	[57]
1.Solid state polymerisation (SSP)	L	Creatinine, CR	PLLA	120000	[58]
2.Melt polymerisation					
1. Melt polymerisation	D	2-Naphthalenesulfonic acid (2-NSA)	PDLA	33300	[50]
2. Solid state polymerisation (SSP)					
1.Ring opening polymerisation (ROP)	L	1.Tin (II)-2-ethylhexanoate, $\text{C}_{16}\text{H}_{30}\text{O}_4\text{Sn}$	PLLA	203000	[59]
2.Chain extension		2.Hexamethylene diisocyanate, HDI			
Enzymatic polymerisation	DL	-	PDLLA	2400	[60]
	L	-	PLLA	4500	[61]

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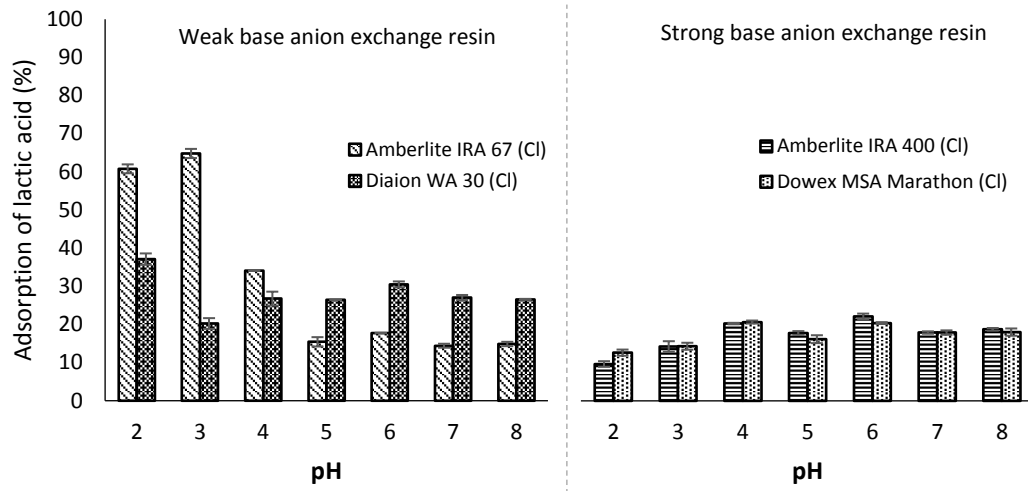
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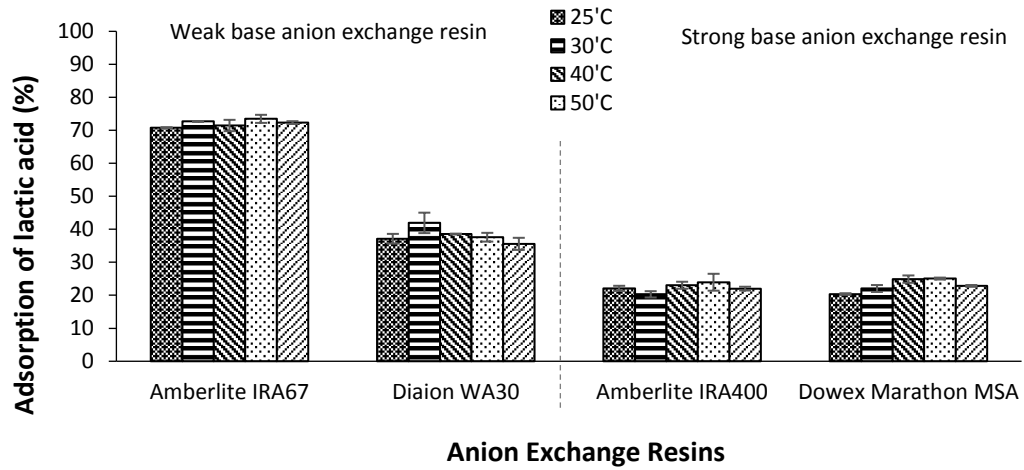
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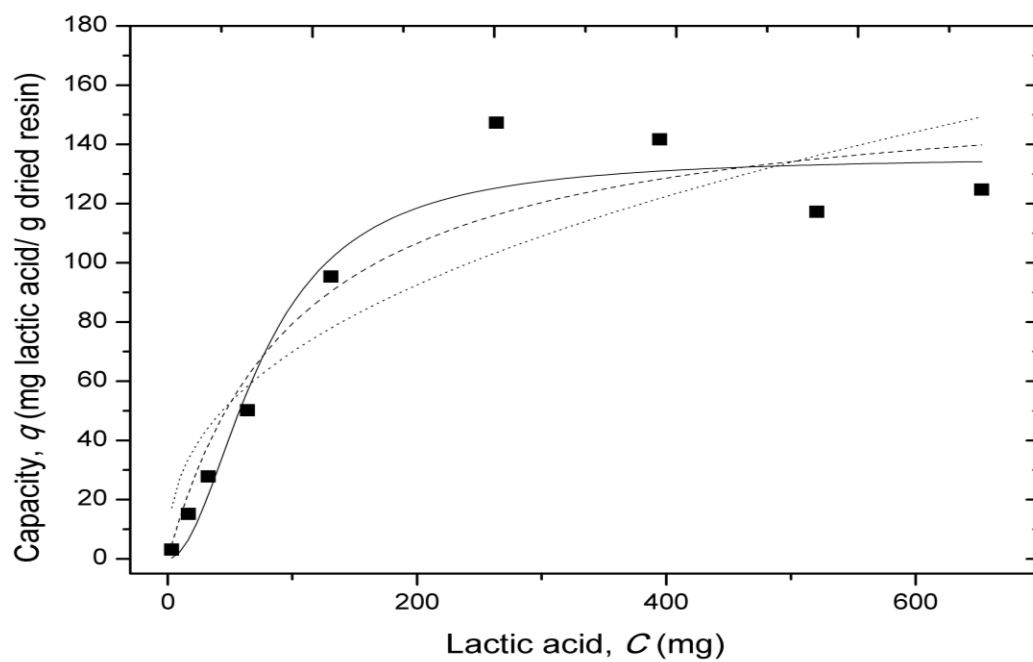
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Figure 6

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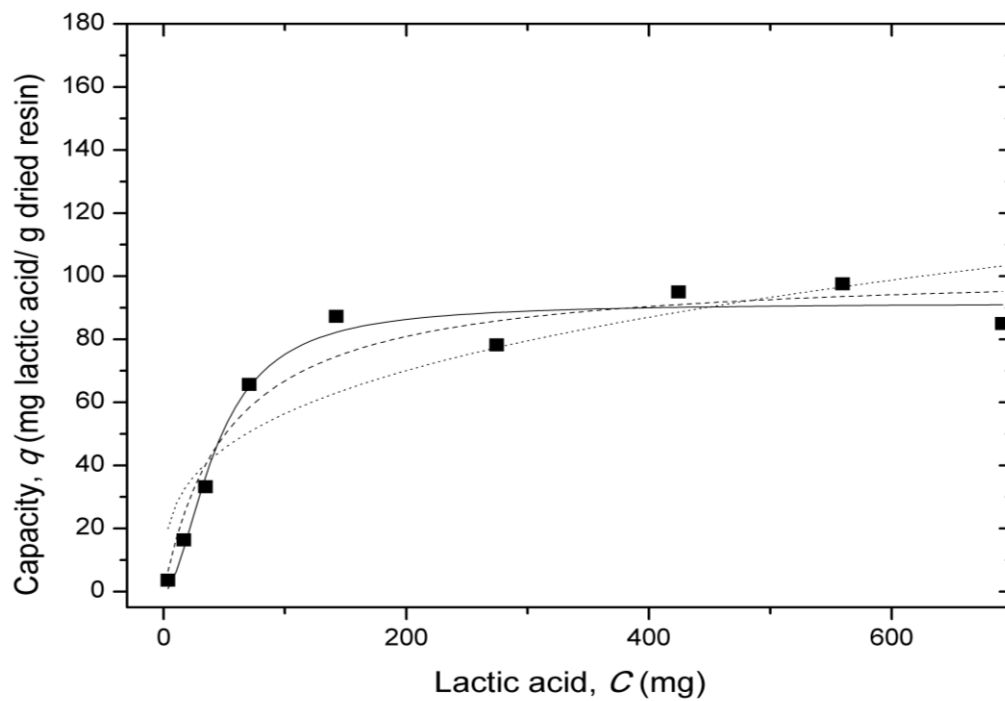
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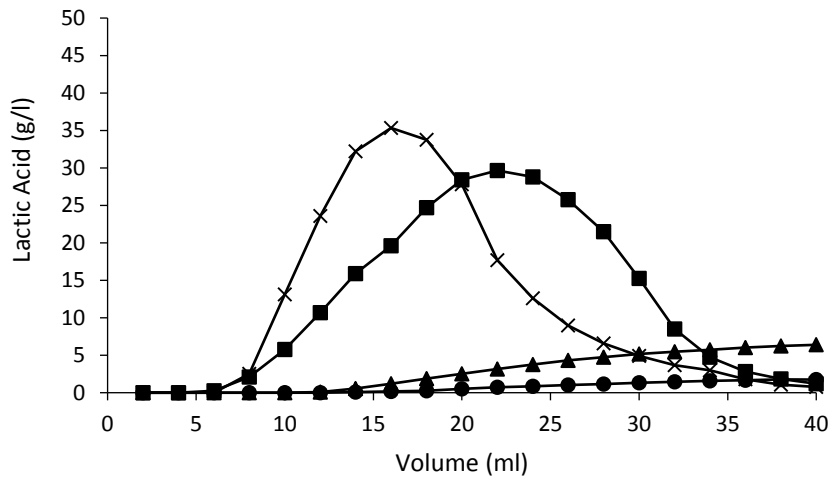
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Figure 7

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Figure 8

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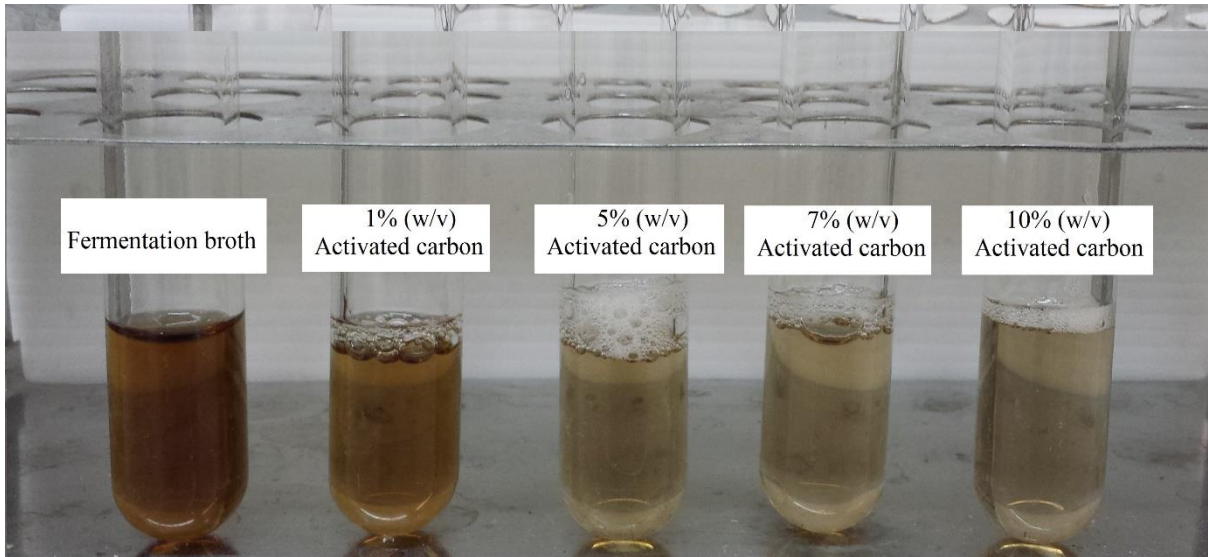
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Figure 9

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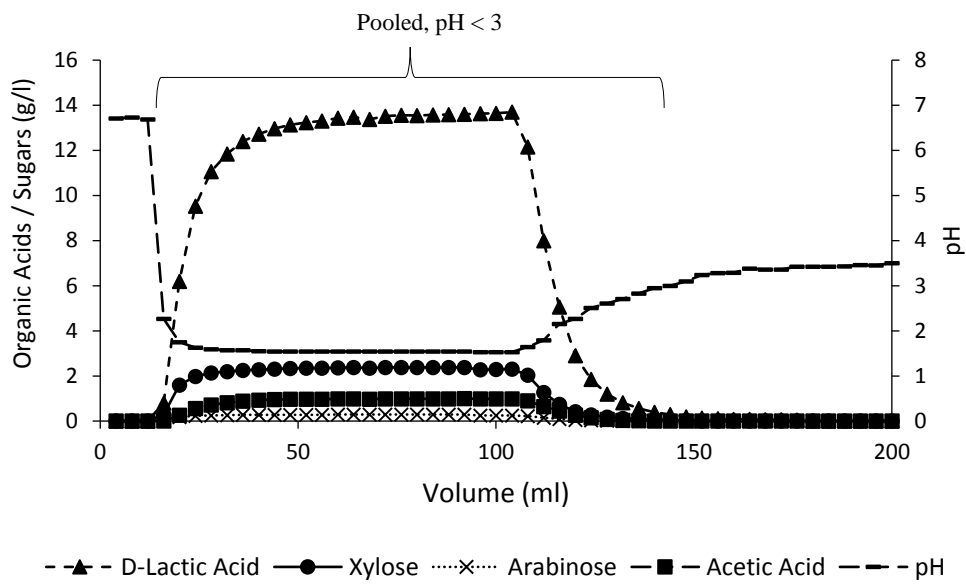
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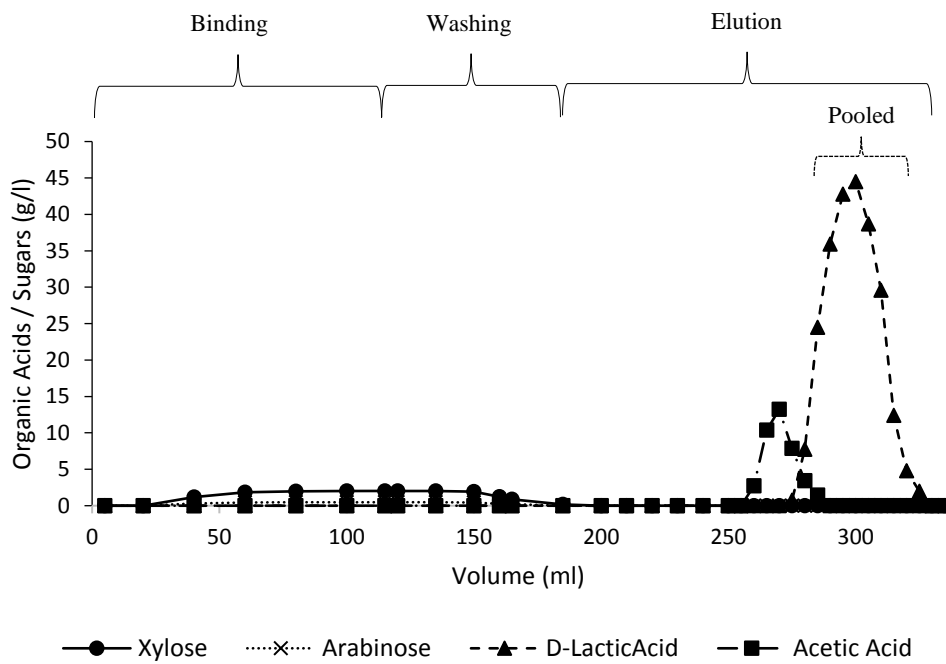
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Figure 10