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Research paper

# Cost-effective valorization of cassava fibrous waste into enantiomerically pure D-lactic acid: Process engineering and kinetic modeling approach

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**Abstract**

Cassava fibrous waste (CFW) valorization on the synthesis of D (-) lactic acid (DLA) holds enormous importance, particularly in the production of thermostable and biodegradable polymers. In this study, microbial kinetic modelling was carried out to investigate the dynamics of cassava fibrous waste enzyme hydrolysate (CFWEH) utilization towards DLA production. Designed biomass approach was attempted to evaluate the natural DLA producing organisms, capable of metabolizing CFWEH into optically pure DLA. *Sporolactobacillus inulinus* (NBRC 13595) was found to be the elite strain, resulting the yield of 99.43 % optically pure DLA using CFWEH-supplemented medium. Yeast extract (2 $gL^{-1}$) was observed to be potential nitrogen source over other complex nitrogen sources for kinetic modelling investigation. Kinetic parameters predicted from the proposed model for DLA production showed maximum specific growth rate, $\mu_{max}$ - 0.36 ($h^{-1}$); growth-associated product coefficient ($\alpha$ = 0.47 $gg^{-1}$) and specific productivity ($q_{P,max}$ = 1.12 $gg^{-1}h^{-1}$) respectively. Experimental data of biomass growth, substrate consumption and DLA production with initial sugar concentrations ranging from 20 – 180 $gL^{-1}$ was found to be synchronized well with the simulated dynamic profiles. Kinetic investigation reported in this study is a novice attempt enumerating the valorization potential of CFW for the synthesis of value-added products including DLA at commercial scale in near future.

**Keywords:** Cassava fibrous waste, Waste valorization, Designed biomass approach, *Sporolactobacillus inulinus*, Optical purity, Kinetic modeling.

**Nomenclature:**

| | |
|---|---|
| X | Biomass concentration ($gL^{-1}$) |
| S | Substrate concentration ($gL^{-1}$) |
| P | DLA concentration ($gL^{-1}$) |
| $\mu_{max}$ | Maximum specific growth rate ($h^{-1}$) |
| $K_{ix}$ | Substrate inhibition constant for growth of biomass ($gL^{-1}$) |
| $K_{sx}$ | Substrate limitation constant for growth of biomass ($gL^{-1}$) |
| $P_{ix}$ | Threshold DLA concentration for growth of biomass ($gL^{-1}$) |
| $P_{mx}$ | Maximum DLA concentration for growth of biomass ($gL^{-1}$) |
| $K_d$ | Death rate constant ($h^{-1}$) |
| $q_{s,max}$ | Maximum specific sugar utilization rate ($gg^{-1}$ $h^{-1}$) |
| $K_{ss}$ | Substrate limitation constant for CFWEH consumption($gL^{-1}$) |
| $K_{is}$ | Substrate inhibition constant for CFWEH consumption($gL^{-1}$) |
| $P_{is}$ | Threshold DLA concentration for CFWEH consumption($gL^{-1}$) |
| $P_{ms}$ | Maximum DLA concentration for CFWEH consumption($gL^{-1}$) |
| $q_{p,max}$ | Maximum specific DLA production rate ($gg^{-1}$ $h^{-1}$) |
| $A$ | Growth-associated constant in Luedeking–Piret model ($gg^{-1}$) |

| | |
|---|---|
| $K_{sp}$ | Substrate limitation constant for DLA production($gL^{-1}$) |
| $K_{ip}$ | Substrate inhibition constant for DLA production($gL^{-1}$) |
| $P_{ip}$ | Threshold DLA concentration for DLA production($gL^{-1}$) |
| $P_{mp}$ | Maximum DLA concentration for DLA production ($gL^{-1}$) |
| $Y_{p/s}$ | DLA yield on CFWEH consumption ($gg^{-1}$) |
| $Y_{x/s}$ | Biomass yield on CFWEH consumption ($gg^{-1}$) |
| $R^2$ | Correlation coefficient (dimensionless) |
| T | Fermentation time (h) |
| $S_0$ | Initial substrate concentration ($gL^{-1}$) |

## 1. Introduction

Biodegradable polymers are obtained from starch/cellulose based agricultural feedstocks and food/beverage industry waste either through microbial fermentation directly or by polymerization of the fermentation-derived monomers (Koller et al., 2017; Liang and Wan, 2015; Reddy Tadi et al., 2017; Solaiman et al., 2006). Few examples include synthesis of Poly (Lactic Acid) (PLA), Polyhydroxybutyrate (PHB) and Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV), a co-polymer of PHB through metabolic routes of various organisms (Jem and Tan, 2020; Jyothi et al., 2005; Moorkoth and Nampoothiri, 2016). PLA is a biodegradable polymer possessing extensive advantages in terms of tensile strength, durability and relatively lower production economics at par with any petrochemical-based polymers.

PLA application in the package industry is driven by several factors viz. readily biodegradable, efficient municipal waste management and Food-grade package material. D-lactic acid (DLA) is a monomer of PLA and remains an important platform chemical, its versatile applications suit well for chemical, pharmaceutical, agriculture, textile, and plastic industries (Djukić-Vuković et al., 2019). Synthesis of thermostable polymer, poly D (-)/L (+) lactic acid (PDLLA) opened up new avenue for futuristic applications, which is obtained by blending controlled ratio of poly L (+) lactic acid (PLLA) with poly D-lactic acid (PDLA) (Jem and Tan, 2020). PDLLA has improved crystallinity and enhanced melting point from 180$^{o}$C to 230$^{o}$C compared to poly lactic acid (PLA) obtained from optically pure isomers (DLA or L (+) lactic acid (LLA)) alone or racemic mixture (Fukushima et al., 2007). According to lactic acid market analysis report 2019-2025 (Grand view research, Inc., USA), the estimated global lactic acid market in 2019 was 3.1 billion USD and would reach 8.7 billion USD by 2025 (Crops et al., 2019). The worldwide PLA production capacity was estimated to be 375,000 tons by 2020 whereas its market demand would be around 500,000

tons by 2021 (Jem and Tan, 2020). Industrial production of DLA can be achieved either through conventional chemical route or microbial fermentation. The petrochemical based chemical process yields racemic mixture, whereas microbial fermentation by Lactic acid bacteria (LAB) synthesizes enantiomerically pure D (-)/L (-) lactic acid (Wee et al., 2006).

Economical production of value-added compounds by valorization of 2$^{nd}$ generation agricultural feedstocks say corn cobs, wheat straw, sugar cane waste, barley husk, etc are advantageous not only for cost-effective production process but also benefits from environmental friendly perspective (Ayodele et al., 2020; Nwamba et al., 2021; Pleissner et al., 2016; Saini et al., 2015; Wietschel et al., 2019; Kiran et al., 2021). Promoting agro-based feedstocks to synthesize bulk chemicals would cut down the costs of carbonaceous raw materials drastically. Geographical nature of agricultural feedstocks and its characterization about the organization of sugar residues and other impurities would determine its sustainability; qualify for fermentation, followed by the synthesis of a bioproduct. Cassava fibrous waste (CFW) is a sago industrial waste, rich in starch content (40.1 – 75.1 % on dry weight basis) and generated from cassava processing industries during the peeling, crushing and sieving process (Jyothi et al., 2005). Cassava, being one of the staple crop in the sub-tropical region of the world, produces 291,993 $\times 10^3$ tonnes as per FAO Statistics 2019 (A Otekunrin and Sawicka, 2019). The waste generated from cassava processing in both liquid and solid forms have been used for the production of bio-fuels and platform chemicals like ethanol, citric acid, lactic acid etc. (Zhang et al., 2016). DLA is an important platform chemical of potential commercial interest and can be obtained through microbial route by Lactic acid bacterial (LAB) fermentations, which naturally synthesizes the product. This investigation aimed at valorizing the CFW peel wastes by microbial degradation to reduce the overall organic loading in the natural environment. Commercial interests of D-lactic acid (DLA)

vests in the production of stereo-complexed poly D (-)/L (+) lactic acid (PDLLA) as discussed previously. LAB preferentially adopt fermentative route than oxidative TCA cycle conserving more energy and almost all LAB species can be characterized based on its homofermentative nature (Gänzle, 2015). Microbial production of DLA is significantly influenced by the cost of raw material.

Gaining kinetic insight on DLA production from Cassava Fibrous Waste Hydrolysate (CFWH) would be immensely useful in understanding the process behaviour, crucial to optimize, design, control and improve the sustained production of DLA. Several research groups developed the kinetic models for the lactic acid production from renewable feedstocks (Alvarez et al., 2010; Amrane and Prigent, 1999; Nandasana and Kumar, 2008; Sharma and Mishra, 2014). Fermentative lactic acid production was found to be inhibited at high substrate and product concentrations, which lead to reduction of overall product yield and productivity.

This present study is a novice attempt involving screening the process factors (e.g., elite strain and nitrogen sources) by Designed Biomass Approach (DBA) (Zhao et al., 2019). Major advantage in employing DBA is that simpler selection of existing wild type strains suitable for the utilization of the processed raw feedstock than genetic manipulation. It is practically easier for selection, can be assessed based on higher product titer, yield and other quality attributes like optical purity etc. Following the organism selection, kinetic investigation of experimental data using different unstructured models for DLA production from CFWH was dealt in this study. The kinetic model parameters reported in this study would be of great significance in addressing the technical bottlenecks for a sustained DLA production. In a nutshell, the proposed study of waste valorization, followed by kinetic model and its impact can be explained in the schematic figure (Fig. 1).

## 2. Materials and Methods

### 2.1. Raw material

CFW was procured from small scale sago industries located around Salem, India. Optimization of acidic and enzymatic digestion of raw CFW improved the efficiency of its overall conversion into utilizable sugars by 2.47 % (w/v) and 7 % (w/v) respectively (Cingadi et al., 2015). Also, optimal concentration of HCl for CFW hydrolysis was determined to be 1.67 M. Enzyme hydrolysate was obtained using α- amylase (12 AGU, M/s Himedia Laboratories, Mumbai, India) for the hydrolysis of linear chain and amyloglucosidase (M/s Richcore Lifesciences Pvt. Ltd., Bengaluru, Karnataka, India) digesting the branched segment of the CFW biomass, respectively. The temperature and pH were maintained at optimized conditions reported (Cingadi et al., 2015).

### 2.2. Organism and culture conditions

Homo-fermentative LAB (HFLAB) organisms used in this study are *Lactobacillus delbrueckii* subsp. *delbrueckii* (NBRC 3534 and 3202 strains), *Sporolactobacillus inulinus* (NBRC 13595), *Sporolactobacillus laevolacticus* (NBRC 102473) and *Sporolactobacillus terrae* (NBRC 101527). All organisms/strains were procured from NITE (National Institute of Technology and Evaluation) Biological Research Center, Japan. Glycerol stocks (30 % v/v) of the cultures were prepared and preserved at -80ºC.

HFLAB strains were grown in MRS (de Man, Rogosa and Sharpe) preculture media. Its ingredients are as follows (in $gL^{-1}$) for 100 mL culture volume: Glucose - 20; Yeast extract- 5; Beef extract - 10; Peptone - 10; Sodium acetate - 5; Di-potassium hydrogen phosphate - 2; Tri-ammonium citrate - 2; Magnesium sulphate heptahydrate - 0.2; Manganese sulphate tetrahydrate - 0.05 and Tween 80. Growth of organism in the preculture media was initiated by an aseptic transfer of preserved stock (organism) into the autoclaved preculture media. 250 mL closed screw

cap bottles were used as culture flasks incubated overnight at 37ºC in a static mode for anaerobic growth. The pH was maintained in a range between 5 – 7 by the addition of $CaCO_3$ (60 w/w % of initial substrate concentration) as a neutralizing agent (Wang et al., 2011). Growth was continued until the cells reach mid-exponential phases (optical density ($OD_{600}$) ≈ 1) and the cells were harvested by centrifuging the culture at 5000 rpm for 10 minutes. The pellet was washed with 0.9 % g/g saline (NaCl) solution and used to inoculate production medium. All the experiments were repeated in duplicates and average of the estimated titer was reported. Standard errors of all the datapoints, from which 95% confidence intervals (CI) were determined by un-paired 2-tailed student's t-tests.

**2.3. Production medium**

Optimal formulation of the production medium composition remains same as preculture medium except two modifications:

(i) Glucose in standard MRS medium was replaced with the Acidic/Enzymatic hydrolysate of CFW (CFWH) as carbon source. Henceforth, the production medium (CFWH + MRS) can be termed as Cassava Fibrous waste substituted media (CFWSM);

(ii) Concentration of selected nitrogen source in CFWSM was varied based on one factor at a time (OFAT) approach.

**2.4. Screening of elite strain**

The HFLAB strains were grown in CFWSM and the initial concentration of CFWH was maintained uniformly at 20 $gL^{-1}$. Inoculated with the glycerol stocks, the prepared static flasks were incubated overnight at 37ºC. The sample collected at the end of the batch was analysed to estimate the DLA concentration and Optical Purity (OP) towards selection of elite strain. The OP computation establishes the relationship between the concentrations of DLA and LLA by Eqn. 1.

$$OP = \frac{DLA}{DLA + LLA} * 100$$

---Eqn. 1

Elite DLA producer screened from the CFW hydrolysate (Acidic/Enzymatic) exhibiting optimal DLA productivity and higher OP was chosen for the subsequent nitrogen source screening.

**2.5. Screening of nitrogen source by OFAT approach**

Yeast extract, Peptone, Tryptone, and Whey protein hydrolysate were used as nitrogen sources for the screening experiments. Selection process considers importance to the higher DLA productivity and enantiomeric purity. The combined nitrogen sources (beef extract, peptone and yeast extract) originally present in the MRS medium was replaced by 25 $gL^{-1}$ of sole nitrogen sources (Either of Yeast extract, peptone, tryptone, and whey protein hydrolysate) in CFWSM. Static flask experiments intended for the selection of the most appropriate nitrogen source facilitating optimal DLA productivity were carried out. The selected nitrogen source was further varied at different levels by OFAT approach to determine the optimal nitrogen concentration requirement by the elite strain i.e. (1, 2, 4, 12, 20 and 25 $gL^{-1}$). Thus, an elite nitrogen source and its optimized concentration chosen in CFWSM at later stages was employed for DLA production at bioreactor level.

**2.6. Bioreactor experiments**

Batch reactor experiments were conducted in a 3L bioreactor (M/s Applikon Biotechnology, Netherlands) with the different initial CFWH concentration. Autoclaved MRS equivalent CFWSM medium substituted with different initial concentration of CFWH (5 – 180 $gL^{-1}$) served as production medium and elite producer strain was employed. Purging of nitrogen gas for 15 mins to remove the traces of oxygen is an important step before preceding the inoculation of anaerobic

LAB (elite strain). Overnight grown preculture ($OD_{600}$ ≈ 1.0) was transferred aseptically into the reactor to initiate growth and samples were collected at regular intervals from therein. The process temperature and agitation rate were set to 37ºC and 180 rpm respectively. The pH was maintained at 6.8 by addition of the minute pulses of 4 M NaOH and 4 M HCl.

**2.7. Analytical methods**

Collected samples were stored at 4ºC before estimating biomass and metabolite concentrations. Cells were separated from the broth by centrifuging samples at 10000 rpm for 10 min. Supernatant was retained separately and pellet was analyzed for biomass estimation ($OD_{600}$) by UV visible spectrophotometer (Gene Quant 1300, M/s GE Health care, NJ, USA). Measured OD values were converted into dry cell weight (DCW) values by using estimated relationship 1 OD = 0.49 DCW ($gL^{-1}$). Glucose consumption was estimated enzymatically by glucose oxidase – peroxidase method using GOD-POD kit (M/s Tulip Pharmaceuticals, Mumbai). DLA and LLA concentrations were assessed by enzymatic method using D/L Lactic acid assay kit (NYZ Tech assay kit) and OP can be computed from the relationship described previously (Eqn. 1).

**2.8. Kinetic modelling**

Microbial kinetic modelling explains the dynamic behaviour of the culture under given process conditions. Major metabolic activities can be grouped into either of growth, substrate utilization and product formation, which can be explained by the mathematical expressions appropriately. Differential equations for biomass growth $\left({}^{dX}/_{dt}\right)$, substrate consumption $\left({}^{dS}/_{dt}\right)$ and product formation $\left({}^{dP}/_{dt}\right)$ accounting the fermentation processes describes complex biological functions.

**2.8.1. Biomass growth kinetics**

Eqn. 2 addresses biomass growth of a LAB, concerning a steady state balance between specific growth and death rates.

$$dX/dt = (\mu - k_d)X$$

---Eqn. 2

Where μ is the specific growth rate ($h^{-1}$), can be calculated during the exponential growth phase and $k_d$ is cell death rate constant ($h^{-1}$). A well-illustrated monod model explains the relationship between the specific growth rate (μ) and substrate concentration (S). As represented in Eqn. 3, the monod kinetics suits well for the substrate limited growth processes.

$$dX/dt = \left(\frac{\mu_{max} S}{K_s + S} - K_d\right) X$$

---Eqn. 3

Where $\mu_{max}$= maximum specific growth rate ($h^{-1}$), $k_s$= Substrate limitation constant ($gL^{-1}$). Higher substrate and product concentrations are the most important factors influencing microbial growth, especially in synthesizing organic acids. Different models were employed to study the proposed microbial growth with substrate limitation and lactic acid inhibition on various substrates. The model equations representing LAB growth reported by Boonmee *et al* 2003 was adopted for the present investigation on the assumption that non-competitive type of inhibition founds valid at extremely higher substrate concentrations (Boonmee et al., 2003). At higher DLA concentration, the inhibition can be assumed to be in linear manner. Cell death rate was assumed to be negligible and notation, $k_d$ finds no relevance to the proposed outcome. The modified equation governing the biomass growth can be represented as shown in Eqn.4.

$$dX/dt = \mu_{max} * \left(\frac{S}{K_{sx} + S}\right) * \left(\frac{K_{ix}}{k_{ix} + S}\right) * \left(1 - \frac{P - P_{ix}}{P_{mx} - P_{ix}}\right) * X$$

---Eqn. 4

### 2.8.2. DLA production kinetics

Leudeking – Piret (LP) model suggests the DLA production rate dependent upon instantaneous biomass concentration (X) and the specific growth rate (μ) linearly as shown in Eqn. 5 (Luedeking and Piret, 2000).

$$\frac{dP}{dt} = \alpha \frac{dX}{dt} + \beta X$$

---Eqn. 5

Where α and β are the growth associated and non-growth associated constants. Modified LP model equation contains substrate limitation, inhibition constants as well as DLA inhibitory terms. The model was represented in Eqn. 6

$$\frac{dP}{dt} = \alpha \frac{dx}{dt} + q_{p,max} * \left(\frac{S}{K_{sx} + S}\right) * \left(\frac{K_{ip}}{k_{ip} + S}\right) * \left(1 - \frac{P - P_{ip}}{P_{mp} - P_{ip}}\right) * X$$

---Eqn. 6

### 2.8.3. Substrate consumption kinetics

In general, substrate consumption gets channelled towards biomass growth, product formation and for the maintenance of the cellular activities/turnover processes. The mathematical form of substrate utilization kinetics is given by Eqn. 7.

$$\frac{dS}{dt} = -\frac{1}{Y_{X/S}} \frac{dX}{dt} - \frac{1}{Y_{P/S}} \frac{dP}{dt} - m_s X$$

---Eqn. 7

Where, $Y_{X/S}$ & $Y_{P/S}$ are respective yields of biomass and product per gram of substrate utilized, $m_s$ is the cell maintenance coefficient. Boonmee *et al* 2003 and Nandasana *et al* 2008 reported a variation in yield at different initial substrate concentrations in the batch production of LA (Boonmee et al., 2003; Nandasana and Kumar, 2008). Under this condition, Eqn. 7 is unlikely to be applied for studying substrate consumption kinetics. To describe the substrate utilization, the Eqn. 8 can be employed.

$$\frac{dS}{dt} = q_{s,max} * \left(\frac{S}{K_{ss} + S}\right) * \left(\frac{K_{is}}{k_{is} + S}\right) * \left(1 - \frac{P - P_{is}}{P_{ms} - P_{is}}\right) * X$$

---Eqn. 8

**2.8.4. Kinetic parameters estimation**

Batch reactor runs operated at different initial substrate concentrations yielding offline biomass, substrate and product concentrations. The data thus obtained was subjected to determine kinetic parameters, in which minimization of residual sum of squared errors (RSS) between model predicted data and experimental data was obtained from the following equation (Eqn. 9) (Guerra, 2014).

$$RSS = \sum_{i=1}^{n} (X_{model} - X_{exp})^2 + \sum_{i=1}^{n} (S_{model} - S_{exp})^2 + \sum_{i=1}^{n} (P_{model} - P_{exp})^2$$

---Eqn. 9

Where, $X_{exp}$ = experimental value, $X_{model}$ = model predicted value and 'n' is the number of data points. All numerical calculations for modified Monod equations involving substrate inhibitory

terms (Table for different substrate inhibition models) were performed in Microsoft Excel (Ver. 14.7.7) solver add-in (Microsoft Inc, USA). The numerical calculations for solving the non-differential equations were performed using MATLAB R2014a version 8.3 (M/s Math Works Inc., Massachusetts, USA). Correlation (regression) coefficient was determined using the statistical analysis program, StatPlus: mac LE (Analyst Soft Inc.) for Macintosh Operating System. From MATLAB tools ODE 23S solver and 'fmincon' optimization tool has been utilized for resolving complex differential equations of the model. Simulated X, S, P values were derived by minimizing objective function and plotted against time and compared with their corresponding offline datasets. ODE 23S solver is more efficient to solve complex differential equations with appreciable error tolerance.

## 3. Results

### 3.1. Screening experiments

#### 3.1.1. Selection of elite strain and suitable carbon source

DBA was adopted in order to assess the performance of HFLAB strains utilizing CFWH based on final DLA titer, $Y_{P/S}$ and OP (Table 1). Outcomes were promising for the enzymatic hydrolysate (CFWEH) and LAB strains positively responded by completely utilizing the substituted carbon except for *S. terrae*. Growth and overall metabolic activities of LAB strains were found to be relatively inhibitory for Acid hydrolysate (CFWAH), illustrating lesser DLA productivity and yield. Both the strains of *L. delbreuckii* sub sp *delbreuckii* (NBRC 3202 and NBRC 3534) exhibited absolutely zero growth in CFWAH, proving the presence of inhibitory by-products. DLA titer (19.13 $gL^{-1}$), $Y_{P/S}$ (0.96 $gg^{-1}$) and OP (99.43 %) were found to be significantly high for *S. inulinus* growth in CFWEH among other LAB strains. Therefore, *S. inulinus* was found more optimistic for the subsequent exploration towards large scale DLA production. Also, *S. inulinus*

found to be growing well in static culture conditions. These observations found important that the projected LAB strain is highly robust in CFWSM-based medium.

### 3.1.2. Selection of nitrogen source and optimal concentration

Supplementation of various nitrogen sources viz. YE, Tryptone, Peptone, and WPH for *S. inulinus* growth were proven to be effective for DLA productivity (>18.89gL$^{-1}$) and OP (>99 %) (Table 2). The control run (without supplementing nitrogen source) yielded lower DLA titer (7.05 gL$^{-1}$) illustrating the significance of nitrogen supplemented cultures in DLA production. All the nitrogen sources used in this study were proved to be promising but YE was chosen for subsequent investigation owing to its complex vitamin and mineral content (Izaguirre et al., 2020). OFAT approach in determining appropriate YE concentration supporting DLA yield was represented in Fig. 2. Increase in YE level upto 2 gL$^{-1}$ showed improved DLA productivity later which the final DLA titer remained constant irrespective of increase in YE concentration (Optimal YE concentration ≥ 2 gL$^{-1}$). Hence, *S. inulinus* grown in CFWEH as carbon source and YE (2 gL$^{-1}$) as nitrogen source was considered much suitable for lab-scale cultivation and microbial kinetic studies.

### 3.2. Kinetic modelling

Simple unstructured and non-segregated models were investigated to explain DLA production by microbial fermentation. Monod model serves to be universally applicable to all genera of bacteria and yeast except fewer microbial systems. It reliably explains microbial kinetics of growth, substrate utilization and product formation with suitable modifications interpreting substrate and product inhibition terms (Alvarez-Ramirez et al., 2019). The resolved differential equations 4, 6 & 8 representing growth, DLA formation and CFWEH utilization were plotted with their respective offline values at different substrate concentrations of growth-promoting (Fig. 3) and

inhibitory concentrations (Fig. 4). The kinetic equations enlisted in Table A.1 were evaluated with the offline estimated μ values against their respective CFWEH concentrations to explain respective models and other kinetic parameters. All the models could convincingly explain the overall growth and resolve kinetic parameters ($\mu_m$, $K_S$, $K_I$) were tabulated (Table A.2). The model parameters are highly likely reliable from operational perspective as the regression coefficient, $R^2$ is above 0.99. Relatively higher $R^2$ value (0.9994) and lower RMSE value (0.000712) corresponds to Edward (Tipo-Teisser) model indicates the nature of LAB growth closely allied to the estimated parameters.

**4. Discussion**

Several reports are available for L-lactic acid production from renewable resources at pilot scale level, but DLA fermentation at larger scale was rarely reported (Liang et al., 2020). Even metabolically engineered *Bacillus subtilis* strain by incorporating thermo-tolerant *ldh* gene derived from *L. delbrueckii* had yielded an OP of 98 % (Awasthi et al., 2018). But DBA-based screening resulted in identification of potential DLA producers with highest OP (Above 99 % purity) for most LAB strains investigated in the study. This technique has been found successful in identifying suitable LAB to valorize CFW-based substrate by its natural selection. Yield of DLA (0.96 g/g) and the higher OP (99.5 %) were accomplished under controlled operating conditions at a laboratory scale. Pre-treatment by mineral acid finds more suitable for any lignocellulosic biomass to achieve better recovery of utilizable sugars. But in the present study, acidic hydrolysate did not work for any LAB strains employed except for *S. inulinus*, showing moderate DLA productivity (15.41 $gL^{-1}$) and better OP (98.95 %). Significant DLA synthesis in CFWAH-based medium for *S. inulinus* can be attributed for its higher survival rate in acidic and bile salts medium, also determined to be highly stabler than other LAB organisms (Huang et al., 2007). Also, the evolution

of inhibitory compounds like furfurals and its derivatives during acid hydrolysis in CFWAH were determined from preliminary investigation (Cingadi et al., 2015; Tanaka et al., 2019). Enzymatic hydrolysate does not have such complications. Drastic improvement in DLA productivity (Table-1) when compared to other LAB strains proves that *S. inulinus* can grow invariably and metabolize CFWAH actively in the presence of inhibitory compounds (Bai et al., 2016; Zacharof and Lovitt, 2013). Although organism appears stable in CFWSM containing acidic hydrolysate, but DLA titer was significantly lesser than CFWEH containing medium. Owing to the enzyme specificity (α-amylase and amyloglucosidase) and hydrolysis at optimized conditions, availability of free glucose in the CFWEH was higher than in CFWAH. Also, a major fraction of free glucose in CFWEH was channelized towards product formation and therefore found to be a better substitute as a carbon source in the production medium. *S. inulinus* was also observed to be highly stable for most of the nitrogen sources and obtained results were found to be highly reproducible (Table-2). No drastic change in final DLA titers (18.89 – 19.16 $gL^{-1}$) for various nitrogen sources exhibiting the capability of organism in metabolizing the carbon and nitrogen substrates. YE as a nitrogen source would be highly conducible for reactor studies owing to its rich amino acid and mineral salt content(Nancib et al., 2005). A significant drop in DLA titer (7.05 $gL^{-1}$) for control run exemplifies nitrogen source supplementation is vital for the fastidious LAB growth (Wang et al., 2011). Kinetics of LAB strains are reported and entailed elaborately with different carbon sources and compared in the Table 2.

**4.1. Growth kinetics**

Applying simpler monod growth model to account LAB growth can hardly differentiate the exponential and stationary phases. Therefore, many reports suggest the adequacy of mathematical modelling can be fulfilled by incorporating logistic equation, explaining various phases of growth

more reliably (Rohit et al., 2018; Wang et al., 2020). Invariably the depiction of simulated biomass represented in Fig. 3 & 4 showed a good overlapping with the estimated offline values. A regression coefficient above 0.9 shows good reliability of the model with the experimental results and offers better prediction at the given conditions (Akermann et al., 2021). In this study the regression coefficient, $R^2 > 0.99$ for the proposed logistic equation signifies that the obtained experimental results could be highly reproducible for different initial CFWEH concentrations (Fig. 3 & 4). Table A.2 illustrates the application of different model equations previously developed for the kinetic models were fitted with the respective offline μ and $S_0$ to obtain simulated saturation, $k_s$ and inhibitory concentrations, $k_I$. The obtained/simulated values were found to be more realistic and comparable with the previous kinetic investigations of other LAB organisms as shown in Table 3. Estimated specific growth rate is an important indicator and relates the affinity of an organism to the alternate carbon source formulated in this study. Maximum specific growth rate, ($\mu_m$) determined to be 0.31 $h^{-1}$ by offline and could be convincingly predicted by the deployed models (0.36 $h^{-1}$) between 20 – 45 $gL^{-1}$. Biomass limitation rate, $k_{sx}$ was found to be 0.85 $gL^{-1}$ and in most of the reactor runs yielded a consistent biomass concentration above 1.7 $gL^{-1}$. Death constant, $k_d$ determined by the simulation was found to be 0.01 $h^{-1}$ and insignificant compared to the higher growth rate. From Fig. 5 the effect of inhibition can be understood by a steady decline in μ above 20 $gL^{-1}$. Another important indicator of inhibition is the extended lag phase at higher $S_0$ and much smaller lag phases at lower concentrations, as it can be observed from Fig. 3 & 4. Biomass concentration was much reduced to 1.8 $gL^{-1}$ upon a highest substituted CFWEH concentration of 180 $gL^{-1}$. Unutilized residual substrate concentration was found to be significantly higher at 95 $gL^{-1}$ and 180 $gL^{-1}$ and the rate of substrate utilization and biomass growth was significantly reduced (Fig. 4). These observations confirm that inhibitory substrate concentration was exceedingly

higher at higher substrate concentrations above 80 $gL^{-1}$. From the predicted inhibitory concentration of biomass i.e. $k_{ix}$ at 193.94 $gL^{-1}$ may unlikely to occur for lab scale fermentations. Therefore, the inhibitory effects dealt in this study may be confined to CFWEH and DLA concentrations.

**4.2. Utilization of CFWEH**

Unlike acid hydrolysate (CFWAH), enzymatic digests remain free from additional inhibitory compounds like furfurals [Cingadi et al., 2015]. The determination of CFWEH inhibitory concentration i.e. $k_I$ and its relevance to $\mu_{max}$ value of *S. inulinus* could be helpful for the determination of stability of continuous operation of the process. Results of the model points at 99.59 $gL^{-1}$ as a threshold limit until which the microbial system suits well for the Monod type growth. Substrate inhibition was imminent beyond the critical concentration and proceeds at destabilizing the fermentation process (Burgos-Rubio et al., 2000). Based on our previous study, pre-treated/processed feedstock biomass with sugar residue composition was determined to be lesser than the inhibitory levels (Cingadi et al., 2015). It would remain non-problematic even if feeding can be carried out continuously to meet inherent metabolic requirements more dynamically. The specific substrate utilization rate (1.54 $gg^{-1}h^{-1}$) was found to be lesser when compared to other LAB species viz. *Lactobacillus helveticus* (4.8 $gg^{-1}h^{-1}$) and *Lactococcus lactis* (3.42 $gg^{-1}h^{-1}$) (Boonmee et al., 2003; Øyaas et al., 1996). Also, in comparison with other bacterial genera, say *Enterococcus faecalis* (3.33 $gg^{-1}h^{-1}$) was also found to be lower for sucrose-based feedstock (Nandasana and Kumar, 2008). But *S. inulinus* seamlessly engaged to build up significant biomass, higher DLA productivity and yield (> 0.99 g. DLA/g. DCW) at par with other high yielding LAB strains, despite its retarded utilization rate. This seemed to be advantageous for the strain, as it can transformed into industrial workhorse for consistent DLA production. This can

be confirmed from higher affinity constant, $k_s$ (0.85 $gL^{-1}$) reported for *S. inulinus* in utilizing CFWEH shown previously represents the ability of organism in metabolizing the substrate with more ease. Further it was observed that offline DLA titer showed gradual increment upon increasing CFWEH concentrations and reached maximum to 75 $gL^{-1}$ for the substrate loading at 95 g/L, also achieved product yield of 1.01 g. DLA/g. DCW. Inhibition due to higher substrate concentration was visible beyond this point. As a result, DLA titer was significantly reduced (62.27$gL^{-1}$) at 180 $gL^{-1}$ of initial CFWEH. The impact of CFWH utilization on the DLA productivity would have positive effects on the kinetic parameters of product formation as established by Boonmee et.al 2003. These findings and its justifications are crucial for the interpretation of the subsequent DLA production kinetics.

**4.3. DLA product kinetics**

LP model postulated based on the experimental outcome of recurrent lactic acid production processes [Leudeking and Piret, 2000]. The segregation of growth and non-growth-associated product coefficient was already proven to be a best fit for DLA production (Mis Solval et al., 2019; Sharma et al., 2021)(Reddy Tadi et al., 2017). From the growth and product profiles (Fig. 3), it can be well discerned that DLA production is predominantly a growth associated (Sharma and Mishra, 2014). Evolution of DLA creates acidic efflux in the reaction broth, inhibiting the organism growth beyond reaching threshold concentration. The modified model predicts that the coefficient of growth-assisted DLA production (α) found to be 0.47 $gg^{-1}$, while non-growth term (β) was negligible. The lowered production coefficient concomitant with the lower utilization profile proves that proposed kinetic model was stoichiometrically more appropriate. From the model, *S. inulinus* projects that the maximum overall product coefficient ($q_{P,max}$) was 1.12 $gg^{-1}h^{-1}$. The product coefficient of a raw feedstock at this level is of greater significance in terms of

efficient product conversion. Yield coefficient ($Y_{P/S}$) of most reactor runs accounts 75 – 99 % of the total carbon input and acidic product i.e. DLA competes with regular metabolic machinery at higher product concentrations. An extreme proton gradient generated across the cell membrane necessitates spending of higher maintenance energy drawn from the substituted carbon (Altıok and Tokatlı, 2006). The practical difficulties in carrying out fermentations at higher substrate concentrations can be eased at the incorporation of adding neutralizing agents/buffers. Our previous investigation on *S. inulinus* yielded DLA titer of above 200 g/L metabolizing whey protein hydrolysed medium by incorporating $CaCO_3$ as a neutralizing agent (Reddy Tadi et al., 2017). This investigation exclusively overweighs the sustainable technologies of the future to treat industrial wastes of higher carbon loading. Unstructured, non-segregated model elucidated the dynamic process more reliably to account process level intricacies.

## 5. Conclusion

This study emphasizes the suitability of DBA on valorizing various industrial wastes into a value-added product. LAB strains were successful in utilizing the enzymatic hydrolysate of CFWH and achieving 99 % optical purity and the obtained DLA yield coefficients showed promising outcome. *S. inulinus* exhibited a good track record in adopting to the supplied CFWEH (20 $gL^{-1}$) and YE (2 $gL^{-1}$) as limiting nutrients. Application of the kinetic model encumbered monod/inhibition ranges defined for the synthesis of optimal DLA production. The model simulation data showed very good correlation with the experimental data at different initial substrate concentrations with negligible standard errors. In future, proposed model would be helpful in designing and development of bioprocesses for the sustainable production of optically pure DLA from renewable agricultural waste feed stocks at an industrial scale.

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**Author's contributions**

SS* has acquired and managed funds from CSIR, Govt. of India for the proposed research work, whom with the collaboration of VK* conceptually mentored all the other co-authors jointly for its successful execution. KKG and AEVR jointly carried out the experimental static flask cultures, subsequently operated bioreactor for kinetic modelling and performed all analytical assays. Both SRR and NS have been instrumental in data curation, resolving kinetic models and interpreted the results of their simulation. NM has actively coordinated the research team, engaged with all co-authors to facilitate the progress at various levels in the execution of the project. All authors sincerely contributed the drafting of manuscript and declare that all the simulation and experimental data sharing are authorised on request to the corresponding author (SS*) for academic/research interests.

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**Table 1 Screening of microbial strains on CFWEH and CFWAH for production of optically pure DLA**

| Microorganism | Substrate | $Y^{a}_{p/s}$(gg$^{-1}$) | DLA titer (gL$^{-1}$) | $r^{b}_{p}$(gL$^{-1}$h$^{-1}$) | Optical purity (%) |
|---|---|---|---|---|---|
| *Lactobacillus delbrueckii* | CFWEH[c] | 0.93 ± 0.02 | 16.22 ± 0.61 | 0.91 ± 0.01 | 98.24 ± 0.32 |
| *delbrueckii* NBRC 3202 | CFWAH[d] | NG | NG | NG | NG |
| *Lactobacillus delbrueckii* | CFWEH | 0.76 ± 0.05 | 15.62 ± 0.54 | 0.67 ± 0.03 | 93.22 ± 0.27 |
| *delbrueckii* NBRC 3534 | CFWAH | NG | NG | NG | NG |
| *Sporolactobacillus inulinus* | CFWEH | 0.96 ± 0.02 | 19.13 ± 0.43 | 0.89 ±0.03 | 99.43 ± 0.13 |
| NBRC 13595 | CFWAH | 0.97 ± 0.01 | 15.41 ± 0.71 | 0.59 ±0.02 | 98.95 ± 0.51 |
| *Sporolactobacillus terrae* | CFWEH | 0.80 ±0.04 | 14.10 ± 0.47 | 0.59 ± 0.01 | 75.61 ± 0.34 |
| NBRC 101527 | CFWAH | 0.64 ± 0.03 | 8.25 ± 0.22 | 0.34 ±0.03 | 56.64 ± 0.62 |

[a]DLA Yield ($Y_{P/S}$, gg$^{-1}$) was calculated as a ratio of DLA produced (g) to substrate consumed (g). [b]Volumetric productivity (rp, gl$^{-1}$h$^{-1}$) was calculated as a ratio of concentration of DLA produced (gl$^{-1}$) to fermentation time (h). [c]CFWEH – Cassava fibrous waste enzyme hydrolysate. [d]CFWAH – Cassava fibrous waste acid hydrolysate. All the cultures were grown in static condition at 37 °C, NG- No growth.

**Table 2 Screening of nitrogen source for production of optically pure DLA from CFWEH by *S. inulinus* NBRC 13595**

| Nitrogen source | $Y_{P/S}$ (gg$^{-1}$) | DLA titer (gL$^{-1}$) | $r_P$ (gL$^{-1}$h$^{-1}$) | Optical purity (%) |
|---|---|---|---|---|
| Yeast extract | 0.96 ± 0.01 | 19.16 ± 0.53 | 0.48 ± 0.02 | 99.57 ± 0.17 |
| Peptone | 0.95 ± 0.02 | 19.11 ± 0.21 | 0.39 ± 0.02 | 99.75 ± 0.11 |
| Tryptone | 0.94 ± 0.02 | 18.93 ± 0.64 | 0.41 ± 0.03 | 99.50 ± 0.25 |
| Whey Protein hydrolysate | 0.95 ± 0.02 | 18.89 ± 1.01 | 0.41 ± 0.02 | 99.64 ± 0.21 |
| Control | 0.92 ± 0.01 | 7.05 ± 0.77 | 0.13 ± 0.01 | 99.01± 0.13 |

Experiments were performed in static condition at 37°C

**Table 3 Optimum parameter values for the kinetic model of *S. inulinus* NBRC 13595 and their comparison with reported values of lactic acid production using unstructured model.**

| Kinetic parameter | This work | *Lactococcus lactis* **NZ133 grown on lactose** (Boonmee et al., 2003) | *Enterococcus faecalis* **RKY1 grown on molasses**(Nandasana and Kumar, 2008) |
|---|---|---|---|
| **Biomass formation model** | | | |
| $\mu_{max}$ ($h^{-1}$) | 0.36 | 1.1 | 1.6 |
| $K_{sx}$ ($gl^{-1}$) | 0.85 | 1.32 | 0.89 |
| $K_{ix}$ ($gl^{-1}$) | 193.94 | 304 | 167.46 |
| $P_{ix}$ ($gl^{-1}$) | 1.26 | 1.39 | - |
| $P_{mx}$ ($gl^{-1}$) | 27.51 | 49.9 | - |
| $K_d$ ($h^{-1}$) | 0.01 | - | 0.00318 |
| SSE | 0.231 | | |
| RMSE | 0.103 | | |
| $R^2$ | 0.969 | | |
| **CFWEH consumption model** | | | |
| $q_{s,\,max}$ ($gg^{-1}h^{-1}$) | 1.54 | 3.42 | 3.33 |
| $K_{ss}$ ($gl^{-1}$) | 0.56 | 2.05 | 0.1 |
| $K_{is}$ ($gl^{-1}$) | 99.59 | 140 | 303.17 |
| $P_{is}$ ($gl^{-1}$) | 39.74 | 47.1 | - |
| $P_{ms}$($gl^{-1}$) | 66.71 | 95.5 | - |
| SSE | 206.349 | | |
| RMSE | 2.547 | | |
| $R^2$ | 0.979 | | |
| **DLA production model** | | | |
| α ($gg^{-1}$) | 0.47 | 0.39 | 0.26 |
| $q_{p,\,max}$ ($gg^{-1}h^{-1}$) | 1.12 | 3.02 | 3.0 |
| $K_{sp}$ ($gl^{-1}$) | 0.56 | 2.05 | 0.1 |
| $K_{ip}$ ($gl^{-1}$) | 99.59 | 140 | 303.17 |
| $P_{ip}$ ($gl^{-1}$) | 39.74 | 47.1 | - |
| $P_{mp}$($gl^{-1}$) | 66.71 | 95.5 | - |
| SSE | 72.406 | | |
| RMSE | 1.715 | | |
| $R^2$ | 0.985 | | |

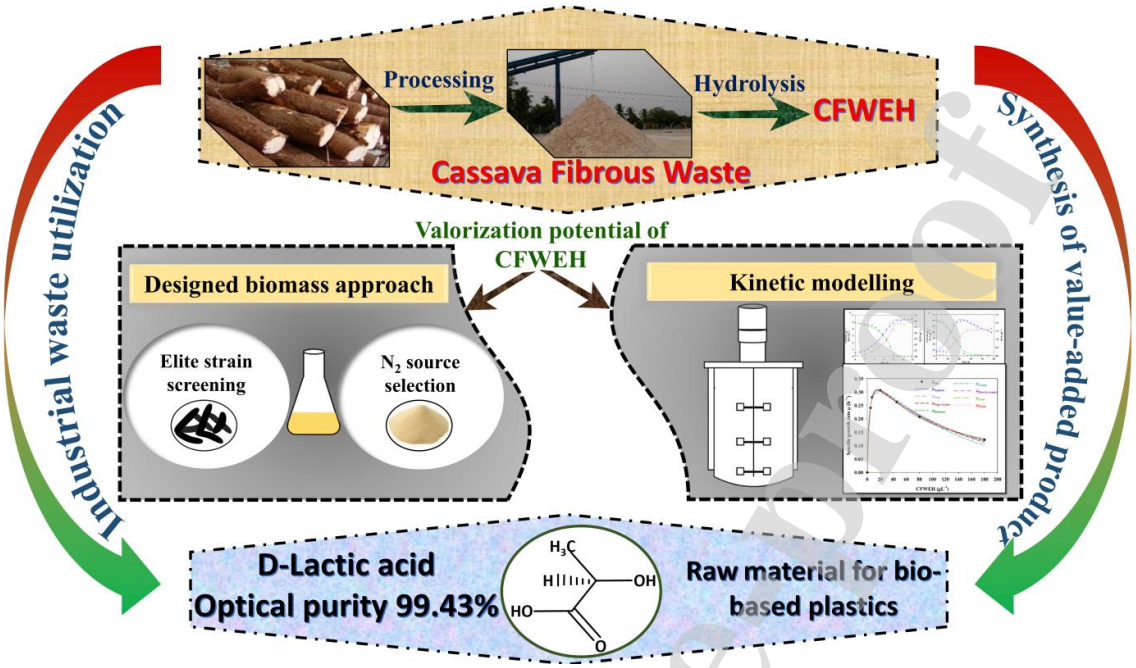


Figure 1. Schematic representation of production of DLA from cassava fibrous waste.

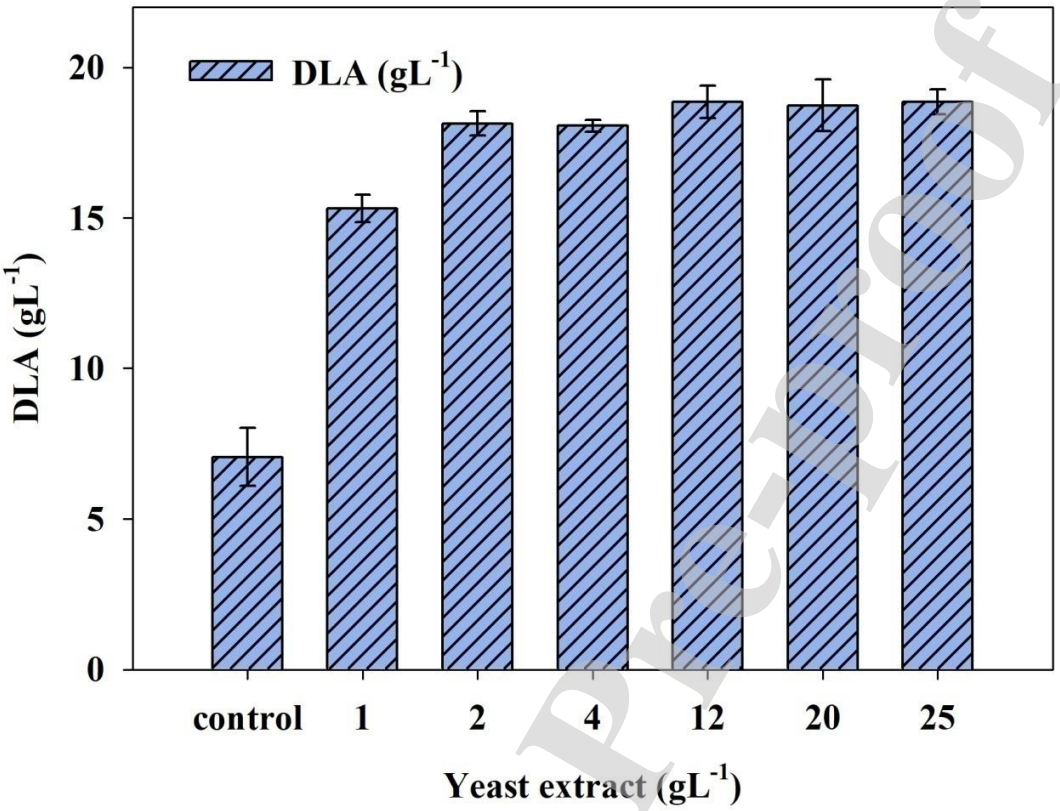


Figure 2. Final DLA titre of the shake flask assessment carried out on varying initial YE concentration using OFAT approach.

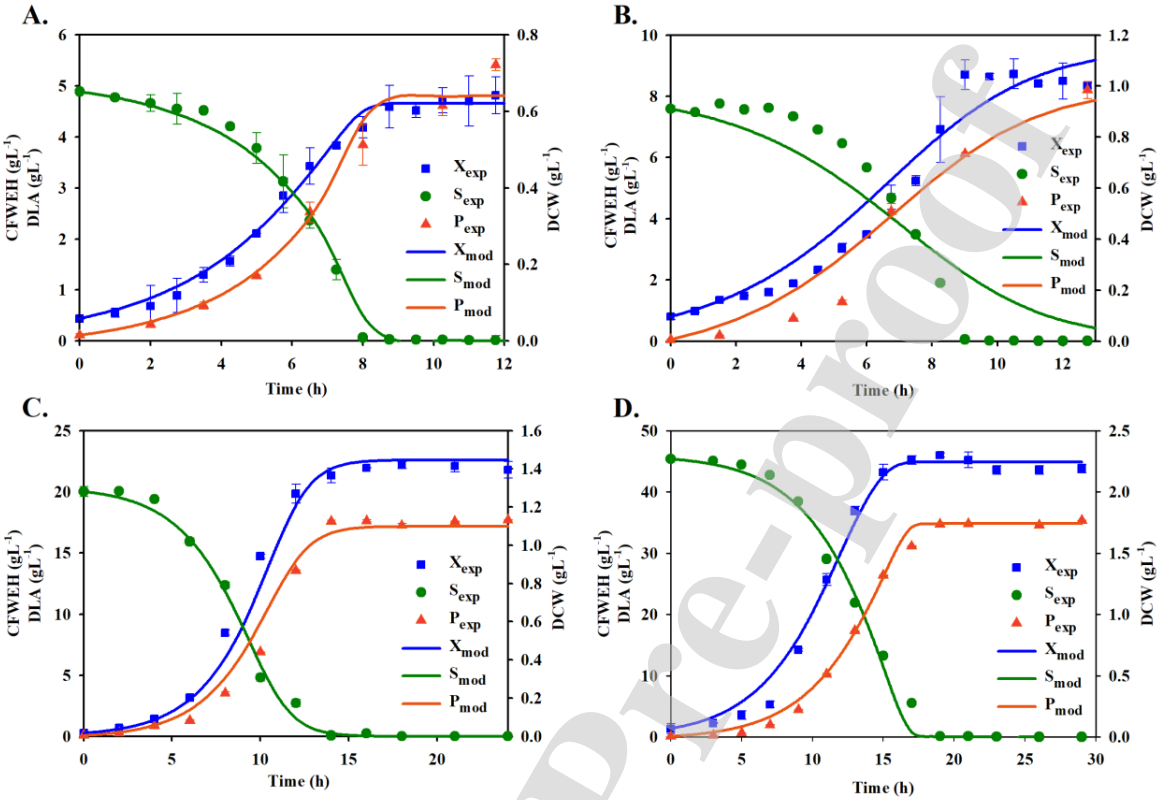


Figure 3. Comparison of experimental profiles of biomass (filled blue squares), CFWEH (filled green circles) and DLA (filled orange triangles) to the respective model predicted values of biomass (blue continuous), CFWEH (green continuous) and DLA (orange continuous) carried out by least square minimization method for different initial CFWEH concentrations: A. 5 $gL^{-1}$; B. 8 $gL^{-1}$; C. 20 $gL^{-1}$ D. 45 $gL^{-1}$.

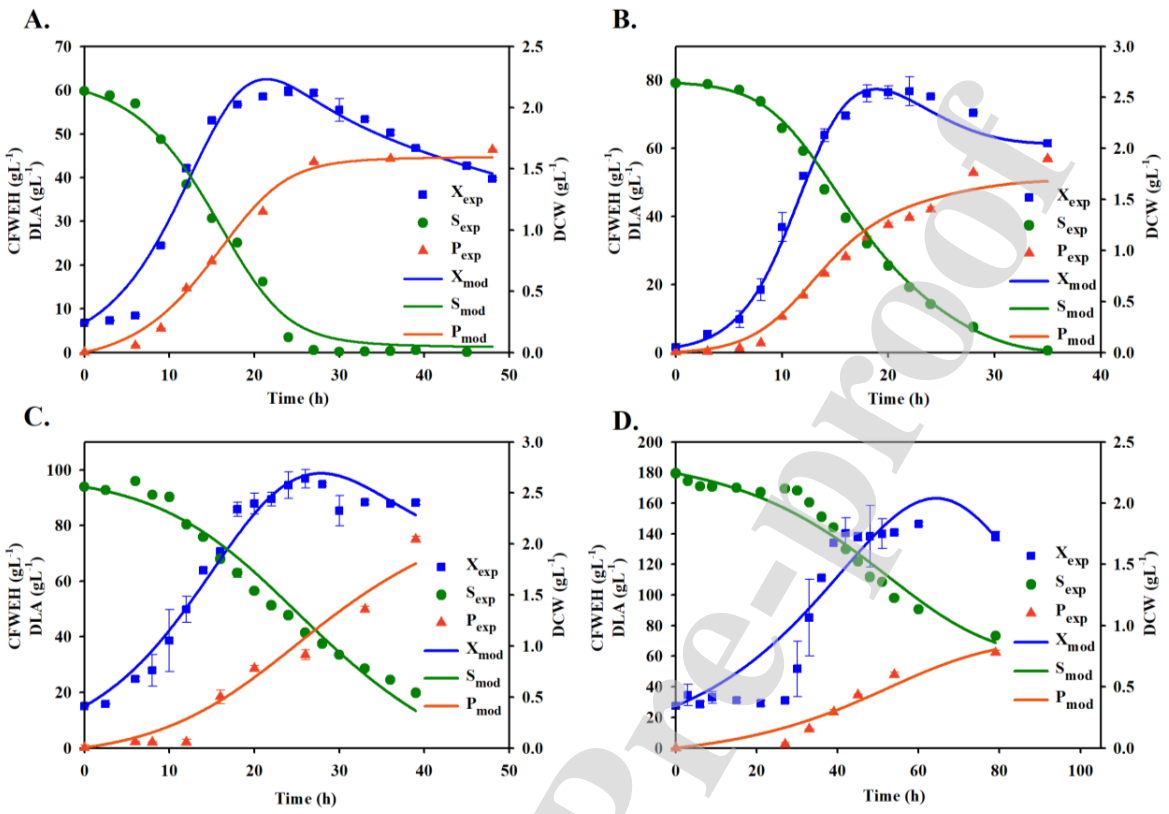


Figure 4. Comparison of experimental profiles of biomass (filled blue squares), CFWEH (filled green circles) and DLA (filled orange triangles) to the respective model predicted values of biomass (blue continuous), CFWEH (green continuous) and DLA (orange continuous) carried out by least square minimization method for different initial CFWEH concentrations: A. 60 $gL^{-1}$; B. 80 $gL^{-1}$; C. 95 $gL^{-1}$; D. 180 $gL^{-1}$.

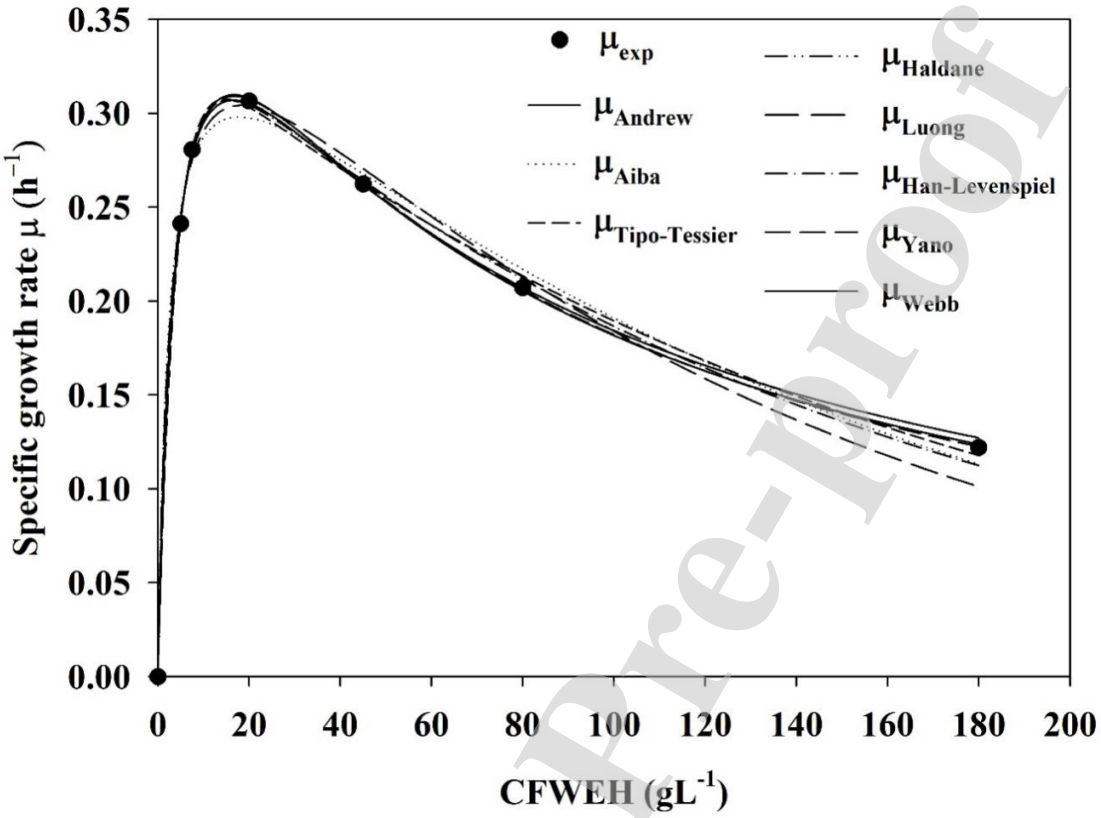


Figure 5. Comparison of the experimental and model predicted specific growth rate profiles for different substrate inhibition models at different initial sugar concentrations.

**Highlights**

- High optical purity (99.43 %) was achieved for D-Lactic Acid
- Valorization potential of cassava fibrous waste was elucidated
- First kind of study on kinetic modeling, utilizing cassava fibrous waste
- Potential scope for techno-economic feasibility of cassava fibrous waste

**Data and material availability**

All the data and illustrations produced in the article can be made available for academic research and teaching upon approval by corresponding author and editor.

**Conflict of interest**

All Authors declare that they have no conflict of interest.