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Fumaric acid production from sugarcane trash hydrolysate using *Rhizopus oryzae* NIIST 1

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Production of organic acids through fermentation of biomass feedstock is a potent strategy for co-product generation and improving economics in lignocellulose biorefinery. Sugar cane trash (SCT), a surplus available agro-residue, was exploited for the production of fumaric acid - a dicarboxylic acid with applications in the synthesis of polyester resins, as mordant and as a food additive. The isolate NIIST 1 which showed the production of fumaric acid was identified as *Rhizopus oryzae*. Media engineering was carried out and a maximum production of fumaric acid in SCT hydrolysate incorporated media was 5.2 g/L. Response surface analyses of the interaction of parameters indicated the importance of maintaining a high C/N ratio. Results indicate the scope for developing the *Rhizopus oryzae* strain NIIST 1 as a potent organism for fumaric acid production, since only a few microorganisms have the ability to produce industrially relevant compounds using lignocellulose biomass hydrolysates.

Keywords: Biomass feedstock, Biorefinery, Lignocellulose, Plackett-Burman design, RSM

Microbially produced organic acids are important market products after antibiotics and amino acids[1]. The organic acid market is growing rapidly and fumaric acid (FA) has been considered as an important biobased building block for future applications[2]. The non-hygroscopic and nontoxic nature of FA attracts its use in various industries. Fumaric acid is a C4 compound with a carbon-carbon double bond and two carboxylic acid groups. The bifunctional nature of the two carboxylic acid groups in FA promotes easy esterification, and the unique structure makes it an ideal source for polymer production, resins, and intermediate compound for various chemical reactions[3]. It is as an effective medication for psoriasis, and also used as an additive in cattle feed[3].

The fumaric acid (FA) is commercially produced by *cis-trans* isomerization of maleic acid in a catalytic process[4]. The malic acid obtained by the hydrolysis of malic anhydride which industrially produced by catalytic oxidation of petroleum derived benzene or n-butane[5]. The increasing price of petrochemicals, high cost of recovery technologies and environmental concerns of the current processes promote biological methods as a sustainable source of FA production. Biological production of FA occurs in the tricarboxylic acid (TCA) cycle of aerobic organisms from malate with the help of a fumarase enzyme. However, the fumarate produced during cell growth mainly consumed for biosynthesis, and thus its concentration cannot build up to a significant amount during growth[4]. In FA-secreting microorganisms, a reductive TCA (rTCA) pathway in the cytoplasm promotes acid production under stress conditions (Fig. 1). Fumaric acid yield through the rTCA pathway varies with different strains and process conditions[6].

Before the petrochemical industry, the fumaric acid (FA) was mainly produced through fermentation. Many

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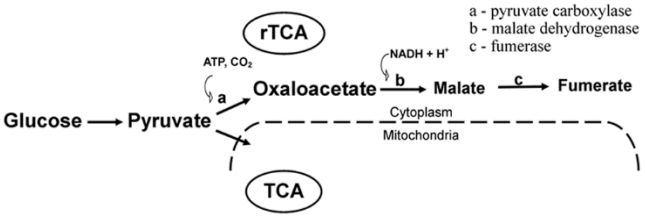

Fig. 1 — Reductive carboxylation pathway in fungus for fumaric acid accumulation

fungal species can synthesize FA, but production at industrially feasible levels reported from only a few species. Industrially relevant fungal species showing FA production mostly belong to *Rhizopus*. The fungal species *R. nigricans*, *R. formosa* and *R. oryzae* have remained the highest producers of FA during fermentation conditions[7-9]. *R. oryzae* is an efficient producer of fumaric acid owing to simple nutrient requirement and lower production costs. The high productivity of FA was reported from *R. oryzae* (4.25 g/L/h) in a glucose medium with rotary biofilm reactor[10].

Fermentative production of FA varies with the different species, nutrient sources, and fermentation conditions. Carbon and nitrogen sources are the key factors affecting the growth and production of FA in microbial fermentation[11]. The parameters like pH, agitation, inoculum size, and fungal morphology influence the production of FA during the industrial fermentation process. Even though batch reactors usually used, the other configurations like biofilm bioreactors, bubble reactors, fluidized, and fixed beds reactors also tried for industrial production of fumaric acid[11]. Large-scale FA production by fermentation has become unfavourable due to the high production cost and makes it economically not feasible. The price of media components is the major factor affecting the FA production cost. Glucose is the most widely used carbon sources and it is readily metabolized by fungi. As the second largest component in medium, various organic nitrogen sources like yeast extract, and inorganic ammonium salts used for the industrial production of FA. The use of low cost media components can significantly reduce the cost of FA production in fungal fermentation.

Recently, the substrates derived from lignocellulosic biomass is used as a low cost feedstock and become popular for fermentation industry[12]. Fungal species which can ferment sugars derived from lignocellulose material is ideal for FA production, as they can significantly reduce the production costs. In this study, sugarcane trash hydrolyzate was used as a carbon source for fumaric acid production by a newly isolated *Rhizopus* sp. The media components for FA production were optimized using statistical design of experiments.

Materials and Methods

Fungal isolation and screening

Partially decomposed vegetable samples collected from local markets and fungal isolation performed by serial dilution and pour plating on Potato Dextrose Agar (PDA). Purified fungal isolates screened for acid production using acid indicator medium (AIM) containing bromocresol green dye. Quantification of fumaric acid production performed after cultivating the selected fungal isolates in potato dextrose broth (PDB) medium for five days and collecting the culture supernatants after centrifugation. The diluted (1:10) samples were filtered through 0.45 μm nylon filters and subjected to HPLC (Prominence UFLC-XR, Shimadzu Co, Japan) analysis. Isolate producing the highest amount of fumaric acid was selected for further studies.

Fungal identification

Fungal isolate showing fumaric acid production was identified through the sequence analysis of the ribosomal internal transcribed spacer (ITS) gene. Genomic DNA prepared and conserved primers were used for amplification of the ITS gene. The primers used were (i) ITS1-5′TCCGTAGGTGAACCTGCGG3′ (ii) ITS4-5′TCCTCCGCTTATTGATATGC 3′. The final concentrations of the reagents in the PCR reaction mix were 1.0 mM $MgCl_2$, 200 μM dNTP, 100 pmol primers, and 50 ng DNA. The PCR reaction conducted with following conditions in a thermal cycler (MJ Mini, Bio-Rad Laboratories, Germany), 94°C for 2 min, 94°C for 1.0 min (35 cycles), 55°C for 1.0 min, 72°C for 2 min, and a final cycle at 72°C for 2 min. PCR product was sequenced and the sequence similarity analyzed through BLAST analysis. The isolate identified from the most similar sequence obtained from the NCBI database. Multiple sequence alignment of test and reference sequences (NCBI GenBank) carried out using the ClustalW2 program. Phylogenetic analysis was performed using the aligned file in MEGA4[13].

Optimization of process parameters for fumaric acid production

For the maximization of fumaric acid production by the selected fungal strain, experiments were performed with the statistical design of experiments in 100 mL of the medium. The first set of experiments conducted for screening different variables and the second set of

experiments for the optimization of significant variables. Design-Expert v7 (Stat-Ease Inc. MN, USA) used for fractional factorial Plackett–Burman[14] and the Response Surface Box–Behnken[15] design matrices.

Identifying significant variables using Plackett–Burman design

Significant medium components were identified through the experiments conducted using Plackett–Burman design[14] with eleven variables in 12 trials. The variables were sugarcane trash hydrolysate concentration, NH_4NO_3, urea, yeast extract, Corn Steep Liquor (CSL), $CaCO_3$, inoculum size, incubation period, initial pH, $MgSO_4$ and $ZnSO_4$ concentration. In the experimental design for fumaric acid production, variables were represented as numerical factors and studied at low (−1) and high (+1) levels.

Optimization of process variables by Response Surface Method

The three most important variables selected from the results of the initial screening were further optimized and the interaction effects between these factors studied by response surface design[15]. A total of 17 experiments performed for this study and the independent factors selected in low (−1), medium (0) and high (+1) levels. The other media components ($CaCO_3$ 5.0 g/L, KH_2PO_4 1.0 g/L, $MgSO_4$ 1.0 g/L and $ZnSO_4$ 30 mg/L) conditions (Initial pH 5.5, Incubation period 5 days) were kept constant.

A quadratic model chosen for the three variables shown here,

$$Y = \beta_0 + \sum_{i=1}^{k} X_i + \beta_{ii} \sum_{i=1}^{k} X_i^2 + \beta_i \beta_j \sum_{i=1}^{k} \sum_{j=1}^{k} X_i X_j$$

where the predicted response is Y, the overall coefficient is β_o, a linear coefficient is β_i, a quadratic coefficient is β_{ii} and the interaction coefficient is β_{ij} and coded values are X_iX_j. Analysis of variance (ANOVA) of the experimental data performed using the Design Expert® (Stat-EaeeInc, MN, USA) software. The model evaluated by checking the determination coefficient (R) and F-test. To verify the model, the experimental responses were compared with the predicted fumaric acid production. Response surface plots created for analyzing the interaction of parameters and finding the optimal quantities of process variables.

Analytical

Samples collected from culture broth after incubation and supernatants recovered after centrifugation. Samples diluted (1:10) with sterile MilliQ® water, filtered and subjected to HPLC analysis (Prominence UFLC-XR, Shimadzu Co, Japan). The concentration of organic acid estimated in HPLC with the Rezex® ROA-Organic Acid H^+ column (300 × 7.8 mm) (Phenomenex, India) at a temperature of 55°C using 0.01N H_2SO_4 as mobile phase, and detected using a photodiode array (PDA) detector. The glucose concentration is estimated by HPLC with the RPM-Monosaccharide Pb+2 column (300 × 7.8 mm) (Phenomenex, India) with a temperature of 70°C and sterile Milli-Q water as a mobile phase. The detection carried out by RI detector. The fungal growth calculated by measuring the dry cell weight of biomass.

Results and Discussion

Fungal isolation for fumaric acid production

Filamentous fungi are well known for their production of valuable organic acids. The natural production of organic acids provides competitive advantages to fungus over other organisms[16]. Filamentous fungi are the main source of organic acids for industrial fermentation. Fumaric acid production reported from several fungal species, but only a few species can synthesize in significant quantities. The major fumaric acid-producing isolates identified were belonged in *Rhizopus*, *Aspergillus*, *Mucor*, *Cunninghamella* and *Circinella* species[17].

A total of 72 distinct fungal isolates were selected and purified. Based on colour change on acid indicator medium and pH after incubation, a total of 10 different isolates were selected for screening fumaric acid production. Out of the 10 isolates, fumaric acid production was observed only in isolate No. 52 (Table 1). Based on the sequence similarity of the ribosomal internal transcribed spacer (ITS) gene, the isolate was identified as *Rhizopus oryzae*. The sequence of *Rhizopus oryzae* and reference sequences from the database (NCBI) were used to construct a phylogenetic tree by the MEGA4 program. The

Table 1 — Screening of fungal isolates for organic acid production

Isolate No.	Colour change	pH after incubation	Fumaric acid production (g/L)
4	yellow	3.0	Nil
8	Pale green	4.0	Nil
18	Yellow	3.0	Nil
30	Yellow	3.0	Nil
32	Pale green	4.0	Nil
41	Yellow	3.0	Nil
52	Yellow	3.0	0.87
65	Yellow	3.0	Nil
66	Pale green	4.0	Nil
71	Yellow	3.0	Nil

phylogeny study was conducted by UPGMA, and the consistency of the phylogram was assessed by bootstrap analysis (Fig. 2). Fumaric acid producing *Rhizopus nigricans* was first identified by Felix Ehrlich in 1911[18]. *Rhizopus* sp. reported as the most efficient producer of fumaric acid under wide range of process conditions. In *Rhizopus* species, *R. nigricans*, *R. arrhizus*, *R. oryzae*, and *R. formosa* reported as efficient producers of fumaric acid in both aerobic and anaerobic conditions[8,9].

Optimization of process parameters

Identification of significant variables using Plackett-Burman design

The species, *Rhizopus arrhizus* was used for the industrial production of fumaric acid since 1940s[19]. Later, the fermentation method was substituted by a petrochemical method due to its higher yield and better economics. However, the fungal fermentation process is getting more interest due to consumer preference for natural products and the need for sustainable processes for future[20]. The economic feasibility of fumaric acid production through fermentation is not as good as chemical processes, and more studies are needed to improve the fermentation process. The use of filamentous fungus in bio-refineries as a catalyst is promising, and their growth on lignocellulose-based substrates gives a chance to exploit cost effective substrates.

The present study used sugarcane trash (SCT) hydrolysate as a carbon source for fumaric acid production. The Plackett–Burman design were used to study the parameters affecting fumaric acid production by *R. oryza*e NIIST 1. The experiments conducted based on Plackett–Burman's design showed wide ranges in fumaric acid production (Table 2). Regression analysis performed on the data and an equation was developed for fumaric acid production as follows:

$$Y = 0.7 - 0.4\mathrm{B} + 0.3\mathrm{D} + 0.41\mathrm{J}$$

where, B, ammonium sulfate; D, yeast extract; and J, corn steep liquor. The F value of 5.59 and the Prob>F value of 0.0230 showed the significance of the predicted model. The effects of variables on fumaric acid production in a given range were detected. The estimated effects of individual variables on fumaric acid production are given in Fig. 3. The concentrations of ammonium sulfate, hydrolysate sugar, and incubation time had a negative influence, indicating that these parameters can be operated at lower levels to

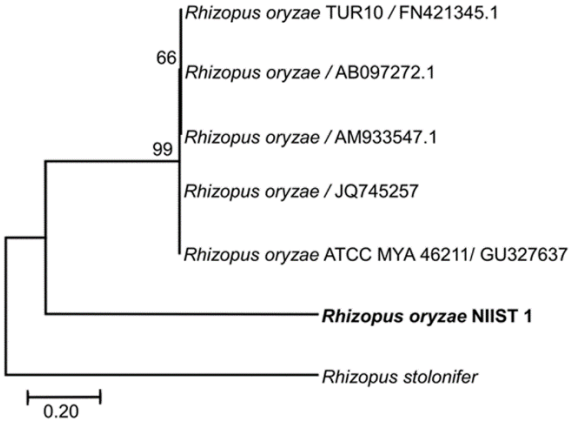

Fig. 2 — Phylogenetic tree expressing the relationships of identified isolate with references

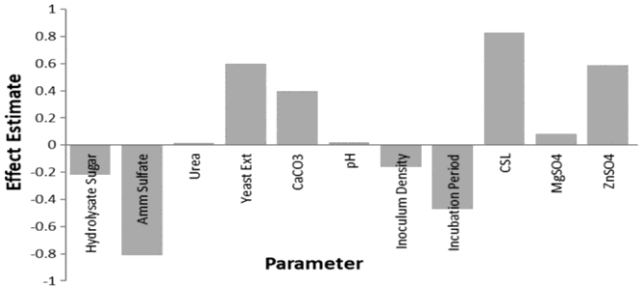

Fig. 3 — Effect of process parameters on fumaric acid production by *Rhizopus oryzae*

Table 2 — Plackett and Burman design matrix and the responses (fumaric acid yields)

Run	SCT Hydrolysate sugar (g/L)	$(NH_4)_2SO_4$ (g/L)	Urea (g/L)	YE (g/L)	$CaCO_3$ (g/L)	pH	Inoculum (bits)	Time (Days)	CSL (mL/L)	Mg SO_4 (g/L)	Zn SO_4 (mg/L)	Fumaric acid (mg/mL)
2	60	2	0	0.5	50	6	5	5	5	0.5	5	0.34
12	60	1	0.2	0.5	20	6	10	7	5	0.2	5	0.32
10	30	2	0	0.5	50	6	10	7	10	0.2	5	0.05
7	30	1	0.2	0	50	6	5	7	10	0.5	5	0.65
9	30	1	0	0.5	20	6	10	5	10	0.5	20	1.15
8	60	1	0	0	50	6	10	7	5	0.5	20	2.22
5	60	2	0	0	20	6	5	7	10	0.2	20	0.49
3	60	2	0.2	0	20	6	10	5	10	0.5	5	0.21
1	30	2	0.2	0.5	20	6	5	7	5	0.5	20	0.00
11	60	1	0.2	0.5	50	6	5	5	10	0.2	20	0.28
4	30	1	0	0	20	6	5	5	5	0.2	5	2.47
6	30	2	0.2	0	50	6	10	5	5	0.2	20	0.27

improve productivity. The concentrations of $ZnSO_4$, yeast extract, and $CaCO_3$ had a positive influence on fumaric acid production. The parameters with significant effects were ammonium sulfate and corn steep liquor with confidence levels above 95%.

The cost of the fermentation medium is a key factor for the commercialization of fumaric acid production, and the use of low cost media is the major focus of many researchers. The present study investigated the potential of sugar cane trash hydrolysate as a carbon source for fumaric acid production. Even though fumaric acid production observed in sugarcane trash hydrolysate, the high concentration negatively affects the production and the low concentration selected for further experiments. The studies conducted using corn straw hydrolysate showed a significant reduction in fumaric acid production with high concentrations of glucose[21]. Nitrogen concentration is another major factor determining the fermentative production of fumaric acid. Ammonium sulfate is the most commonly used inorganic nitrogen source used for fumaric acid[22]. Although the ammonium sulfate is the most commonly used nitrogen source, the ammonium sulfate in this study yielded low concentrations of fumaric acid and a similar result observed in the previous report[23]. The organic source of nitrogen is another option and has some advantages over inorganic nitrogen sources. The studies conducted by Kang *et al*.[23] reported that organic nitrogen source is more effective than inorganic nitrogen sources for fumaric acid production. The different organic nitrogen sources and their concentration have different potential for fumaric acid production. Some studies also reported the combination of organic and inorganic nitrogen sources provided better fumaric acid yield than single sources. The present study also reported the positive influence of organic nitrogen source, CSL, in the production of fumaric acid. The nitrogen content in the CSL is comparable to yeast extract, while the major advantage is the low cost compared to other organic nitrogen sources[24]. Since nitrogen is the second-largest component in the fermentation medium, cheap nitrogen sources like CSL can significantly reduce the cost of production of fumaric acid.

Optimization of process variables using RSM

Based on the results from Plackett–Burman design experiments, the ammonium sulfate concentration and the concentration of corn steep liquor were selected for further optimization. Since high SCT hydrolysate concentration showed a negative effect on fumaric acid production, it was also decided to optimize the levels of carbon source, sugarcane trash (SCT) hydrolysate, in the medium. The two variables with a confidence level above 95% and hydrolysate concentration were optimized, and the concentration of other media components was kept at the levels as specified in published literature (Table 3). The data was analyzed and a second-order polynomial equation was derived as follows:

$$Y = 1.46 - 1.87A - 0.64B - 0.22C + 0.63AB + 0.29AC + 0.12BC + 0.95A^2 - 0.39B^2 - 0.043C^2$$

where the predicted response is Y, and concentrations of hydrolysate (sugar), ammonium sulfate and corn steep liquor (CSL) are represented as A, B and C, respectively. Adequacy of the model tested using Design -Expert software (Table 4). The significance of the model was evident from the F value of 31.3 and a p-value lower than 0.05. The correlation coefficients (R^2) were 0.98, which indicated a better correlation in this study. Table 4 gives the p-values, and more significant variables calculated from their lower p-values. In the present study, hydrolysate and ammonium sulfate (A and B) predicted as significant model parameters. The interaction between hydrolysate concentration and the ammonium sulfate concentration was significant. The interaction of variables and its optimum levels for maximum fumaric acid production evaluated from the response surface curves analysis (Fig. 4).

Table 3 — Box-Behnken experiment design matrix with responses for different trials

Run Order	F1 (%)	F2 (g/L)	F3 (g/L)	R1 (g/L)	R2 (g/L)	R3 (g/L)
1	7.5	0.5	4	1.24	16.56	26.16
2	7.5	1	1	0.57	20.47	16.14
3	10	1	2.5	0.10	20.89	32.93
4	7.5	0.5	1	1.85	15.42	28.41
5	5	0.75	4	3.78	15.98	0.90
6	5	0.75	1	4.88	16.74	0.90
7	7.5	0.75	2.5	1.55	25.00	21.48
8	7.5	0.75	2.5	1.87	20.96	29.05
9	10	0.5	2.5	0.37	20.40	40.39
10	10	0.75	4	0.45	22.28	37.17
11	7.5	0.75	2.5	1.84	16.81	20.10
12	7.5	0.75	2.5	1.14	18.02	24.43
13	5	0.5	2.5	5.19	16.22	0.90
14	5	1	2.5	2.41	17.66	0.90
15	7.5	1	4	0.45	19.41	7.90
16	10	0.75	1	0.39	17.35	46.39
17	7.5	0.75	2.5	0.89	17.43	11.40

[F1-F3, (Factors 1-3= A: Hydrolysate; B: $(NH4)_2SO_4$; and C: CSL), respectively; and R1-R3, (Response 1-3= Fumaric acid production; Biomass; and Glucose left in the medium, respectively]

Table 4 — Analysis of variance for the response surface quadratic model

Source	Sum of Squares	DF	Mean Square	F Value	p value
Model	37.79	9	4.2	31.3	<0.0001
A-Hydrolysate	27.83	1	27.83	207.5	<0.0001
B- $(NH_4)_2SO_4$	3.29	1	3.29	24.5	0.002
C-CSL	0.38	1	0.38	2.9	0.135
AB	1.59	1	1.59	11.8	0.011
AC	0.32	1	0.32	2.4	0.164
BC	0.06	1	0.06	0.4	0.525
A^2	3.84	1	3.84	28.6	0.001
B^2	0.63	1	0.63	4.7	0.066
C^2	0.01	1	0.01	0.1	0.818
Residual	0.94	7	0.13		
Lack of Fit	0.19	3	0.06	0.3	0.799
Pure Error	0.75	4	0.19		
Corrected Total	38.73	16			

The data presented in Fig. 4A indicated a significant interaction between ammonium sulfate and hydrolysate concentration on fumaric acid (FA) production. At lower levels of hydrolysate, an increase in ammonium sulfate not showed any noticeable improvement of FA production while at higher hydrolysate concentration, an increase in ammonium sulfate had a deleterious effect on FA production. The best production of FA recorded at the lowest ammonium sulfate and the highest hydrolysate concentration tested. The interaction between hydrolysate and CSL concentration was not significant, though an increase in CSL resulted in a decreased FA yield (Fig. 4B). The interaction between the organic and inorganic nitrogen sources was also not significant though the increase in either of the nitrogen sources at any given concentration of the other resulted in a reduced FA yield (Fig. 4C). The results indicated for maintaining lower nitrogen content to carbon in the medium for efficient fumaric acid production.

The C/N ratio has a prominent role in fumaric acid production as indicated by the above results and in both cases (Fig.4 A & B), a higher C/N ratio resulted in higher FA production. The C/N ratio has a significant role in the growth and production of various metabolites in microorganisms. The various researchers reported the crucial role of the C/N ratio for fumaric acid production and it was in a range of 120:1 ~ 200:1. It reported that C: N ratios of 120:1 and 150:1 converted 60 and 70% of the carbon source to FA (w/w) in submerged fermentation[25]. A fumaric acid yield of 85% obtained from *R. arrhizus* in a glucose medium with a C: N ratio of 200:1[3]. The influence of

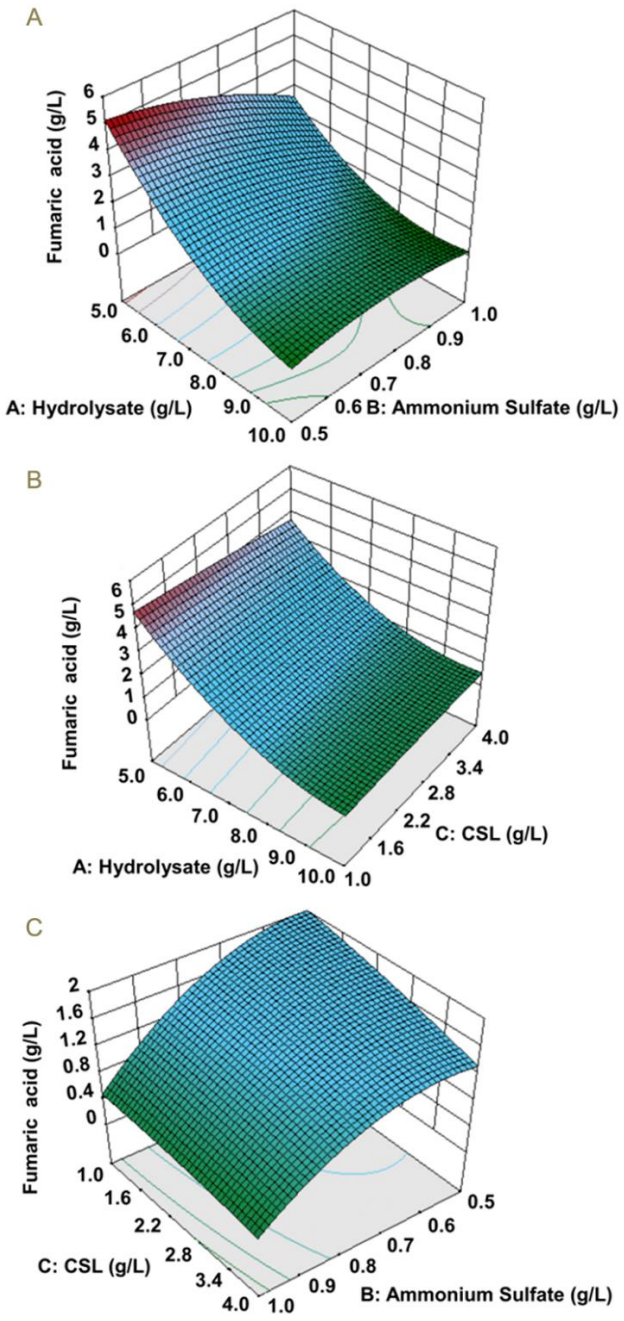

Fig. 4 — Surface plots showing interactions of (A) ammonium sulfate and hydrolysate; (B) hydrolysate and CSL; and (C) ammonium sulfate and CSL concentration during fumaric acid production

the C:N ratio on the FA production investigated in *R. oryzae* and the production was increased by decreasing the concentration of nitrogen source (urea) in the medium[6]. The present study also showed maximum production of FA under the higher C/N ratio in the medium.

Nitrogen source concentration was crucial for controlling the dynamic between the cell growth phase and fumaric acid production in a filamentous fungus. The studies conducted by Zhou *et al*.[26] showed similar results, were nitrogen source regulates the fungal growth and acid production phase in the medium. The fumaric acid biosynthesis in filamentous fungus occurred through a reductive TCA pathway in the cytoplasm. The pyruvate carboxylate enzyme promotes an ATP dependent condensation pyruvic acid with CO_2 to oxaloacetic acid. Oxaloacetate is converted to malate by malate dehydrogenase and malate to fumarate by fumarase in the cytosol[27]. The oxidative TCA cycle in mitochondria and rTCA cycle in cytoplasm compete for pyruvate formed in the glycolysis pathway. The stress is essential for acid production in filamentous fungus and under nitrogen limiting conditions; the cell cannot produce biomass from excess carbon source and switch to acid production[28]. The studies also showed that the nitrogen limitation is a key factor to induce cytosolic fumarase for FA production in *R. oryzae*[6]. When adequate nitrogen was present in the medium, carbon was initially consumed for biomass generation than FA production[29]. In fungal fermentation, when the nitrogen source is fully depleted, FA can be secreted into the medium[19]. Other studies have also revealed that under limited nitrogen conditions, fungi such as *Rhizopus* sp. can accumulate and secrete FA via cytosolic reductive TCA pathway[30,31]. This is evident in our correlation analyses that the FA yield did not indicate any noticeable correlation with biomass. However, it was observed that there is a correlation between FA production and glucose consumption (Fig. 5). Under a higher C/N ratio, after fungal growth, the additional glucose is channelized to acid production.

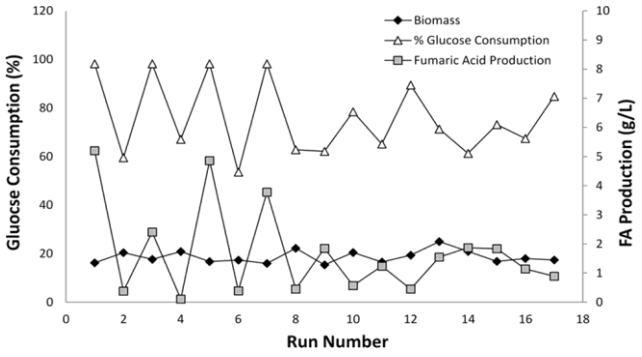

Fig.5 — Graph showing correlation between glucose consumption, biomass accumulation and fumaric acid production

Modern industrial fermentation production systems replace more expensive pure carbon sources by carbohydrate-rich low-cost substrates like molasses, dairy manure, and potato wastes. Lignocellulose is the most abundant biological material for a cheap carbon source in industrial fermentation. Only a few studies are reported for FA production from lignocellulosic materials. Woiciechowski *et al*.[32] reported the FA production using hydrolysate obtained from wood chips after the steam explosion. The maximum production of FA from the best producer, *R. arrhizus* 16179, was around 5.085 g/L and FA yield of 0.31 g/g was obtained with manure fibre hydrolysate supplemented with glucose[33]. Xu *et al*.[21] reported an FA yield of 0.35 g/g with processed corn straw. The highest yield of FA (0.44 g/g) was reported when wood hydrolysate of *Eucalyptus globules* was used as a substrate for production[34]. Fumaric acid production using lignocellulose carbon sources is shown in Table 5.

Conclusion

Microbial fumaric acid production was first noted at the beginning of the twentieth century and continues to be an interesting field of research due to the expanding application of fumaric acid and its derivatives. Fumaric acid production was significantly improved by screening for high-producing microbial strains and the development of efficient bioprocesses. However, the fermentation processes remain unable to compete economically with the chemical methods, and recent research has involved in the selection of raw materials and the development of processes for reducing the cost of production. The ability of microorganisms to utilize

Table 5 — Fumaric acid production using different lignocellulosic carbon sources

Substrate	Strain	Fermentation	Yield	Reference
Wood chips	*Rhizopus arrhizus*	Shake flask	5.08 g/L	32
Cassava bagasse	*R. formosa* MUCL 28422	Shake flask (32°C, 200 rpm)	21.3 g/L	8
Diary manure	*R. oryzae* ATCC 20344	Stirred tank (30°C, 200 rpm, 4 days)	31.0 g/L	33
Corn straw	*R. oryzae* ME-F12 (mutant)	Shake flask (35°C, 200 rpm,96 h)	27.7 g/L	21
Eucalyptus globules wood hydrolysate	*R. arrhizus* DSM 5772	Shake flask (31°C,200 rpm, 32 h)	9.6 g/L	34
Alkali-pretreated corncob (APC)	*R. oryzae*	Shake flask, SSF (38°C, 220 rpm, 60 h)	19.1 g/L	35
Sugarcane trash hydrolysate	*R. oryzae* NIIST 1	Shake flask (30°C, 200 rpm, 5 days)	5.2 g/L	Present study

lignocellulose-derived carbon source opens the possibility of a cost-effective medium for fumaric acid production. The present study reveals the potential of SCT hydrolysate as a carbon source for the production of fumaric acid (FA) by *R. oryzae*. Maximum concentration (5.2 g/L) of FA was reported in a medium containing sugarcane trash hydrolysate (with 5.0% glucose), 0.50 g/L of $(NH_4)_2SO_4$, 2.50 mL/L of CSL, 5.0 g/L of $CaCO_3$, 1.0 g/L of KH_2PO_4, 1.0 g/L of $MgSO_4$, 30 mg/L of $ZnSO_4$ with a pH of 5.5 at 30°C, 200 rpm and an incubation period of 5 days. Response surface analyses of the interaction of parameters indicated the importance of the C/N ratio and the need to keep it high (*i.e.*, low nitrogen levels). So far, sugarcane trash hydrolysate has not been exploited for fumaric acid production. The results of the study suggested that sugarcane trash hydrolysate could be a cheap carbon source for fumaric acid fermentation. The novel fermentation strategies are also essential to scale up microbial fumaric acid production at an industrial scale. Solid-state fermentation is an unexploited area for fumaric acid production and offers higher productivity over submerged fermentation. The fungal immobilization enhanced production of fumaric acid from *Rhizopus* sp. and novel immobilized bioreactor systems can support the scale-up of fumaric acid production with low-cost production medium.

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Conflict of Interest

Authors declare no conflict of interests.

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