RESEARCH ARTICLE

Optimization and kinetic parameter estimation of bioethanol production from freely available Sri Lankan fruits in batch fermentation

I.W. Kularathne, A.C. Ratnaweera, C.S. Kalpage, S. Rajapakse and C.A. Gunathilaka

Highlights

- Sri Lankan overripe fruits: banana, papaya, and jackfruit were used to produce bioethanol.
- Fermentation parameters were optimized by changing the type of fruits, type of microorganism, concentration of the substrate, pH, and temperature.
- Monod and Modified Gompertz equations were used to optimize the kinetic parameters.
- 13 vol% of highest bioethanol yield was recorded for banana fruit with *Pseudomonas mendocino* microorganism at 1:1 (w/w) concentration, pH 5 and 35 °C .

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RESEARCH ARTICLE

Optimization and kinetic parameter estimation of bioethanol production from freely available Sri Lankan fruits in batch fermentation

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Abstract: Bioethanol is used in many countries as an alternative to gasoline, mainly due to better emission characteristics. Since Sri Lanka imports fuel for its whole transportation requirement, this research aims optimization and kinetic parameter estimation of bioethanol production from freely available Sri Lankan fruit wastes. Bioethanol production was carried out using three different freely available overripe fruits in Sri Lanka: banana (*Musa* spp., embul kesel variety), papaya *(Carica papaya)* and jackfruit (*Artocarpus heterophyllus)*. Kinetic evaluation of the fermentation process and optimization of bioethanol yields were carried out by changing the type of fruits, type of microorganism (*Saccharomyces cerevisiae* and *Pseudomonas mendocino* (PM)), concentration of the substrate (1:1, 1:1.5, 1:2 w/w ratio of fruit and water), pH (4.3, 5.0, 5.7), and temperature (27, 32, 35 ^{o}C). Estimation of the kinetic parameters was carried out using Monod and Modified Gompertz equations by fitting experimental data. The best parameters found for the fermentation of banana using PM microorganism were 1:1 (w/w) concentration at pH 5 and 35 oC with a maximum bioethanol yield of 13 vol%. Experimental data were well fitted with Monod model and Gompertz model, with a higher regression coefficient of R2 99.81 and R2 > 99.37 respectively. Comparative to the reported, 12.8 vol% bioethanol yield from banana by fermentation using PM microorganism at room temperature a higher bioethanol yield of 13 vol% could be obtained by fermentating banana fruit mass using PM microorganism with 1:1 (w/w) concentration at pH 5 and 35 ^{o}C temperature.

Keywords: Fruit wastes; Batch-fermentation; Biofuel; Kinetic Modelling; Optimization.

INTRODUCTION

Bioethanol has been identified as an attractive alternative biofuel source for fossil fuel due to several merits such as the possibility of using bioethanol as a transportation fuel, reduction of its greenhouse gas emission, the availability of various renewable feedstocks, etc. Presently, bioethanol is produced from sugar-containing feedstocks such as sugar cane, molasses, etc., starch-containing grains; corn, wheat, etc., lignocellulosic materials such as agricultural residues, wood, etc. and waste materials: municipal waste, vegetable waste, fruit wastes, etc. (Goyal *et al*., 2008; Manochioa *et al*., 2017; Mustapa *et al*., 2008). Bioethanol, blended with gasoline (less than 10 % of ethanol) is widely used in many countries as a transportation fuel with existing gasoline engines because of improved emission characteristics (Mustapa *et al*., 2008).

Nevertheless, owing to the high production cost of ethanol, many researchers have focused more on investigating into freely available efficient bioethanol substrates and microorganisms for higher bioethanol yield (Faradiella *et al.,* 2017). Overripe fruit mass (obtained from waste fruits) has been identified as an ideal substrate for bioethanol production.

Many fruits are well grown in Sri Lanka. Out of the most available fruits, banana, papaya, and jack fruits have been identified as the most sugar-rich fruits which could be used efficiently for bioethanol production (Kularathne *et al*., 2016). Overripe fruits have been identified as proven feedstocks to produce bioethanol by fermentation using *Saccharomyces cerevisiae* since those fruits contain simple sugars such as sucrose, fructose, maltose as well as reducing sugars such as glucose, xylose etc. (Jahid *et al*., 2018). However, fruit wastes such as peels which contain cellulose are with a great constraint to access for the production of fermentable sugars. Such lignocellulosic materials should be subjected to pretreatments such as physical, chemical and biochemical pretreatments before the fermentation process (Behera *et al*., 1996).

Carbohydrate is available in three types as sugars, starches and fibres. Starches are considered as the complex sugars. Unripe fruits mainly contain starch. The amount of starch content of fruits varies depending on the type of fruit and the ripen stage. At the harvest, fruits contain a high percentage of starch and low percentage of sugar. Comparative to other fruits, unripe banana contains 20% starch and 1% sugar at the time of harvest. As the fruits ripen, formation of sucrose takes place first, however, nearly a constant amount while the amount of fructose and glucose content increases until the end of the ripening processes. A fully ripped banana, which is yellow with

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some brown spots, contains 14% fructose, 20% glucose and 66% sucrose (Macrae *et al*., 1992). .

Fruit ripening process takes place in association of transient respiration. It involves a series of events including changes in physiological and biochemical levels resulting in softening, sweetening, decreased bitterness, colour alterations, etc. The major changes can be categorized into three main stages such as starch degradation, tricarboxylic acid (TCA) cycle, glyoxylate cycle, sucrose synthesis and degradation (Kumar *et al*., 2019).

Starch is composed of amylose and amylopectin. During ripening, starch is broken down into simple sugars. Various hydrolytic enzymes such as α-amylase, β-amylase, debranching enzyme, α-1 -4 glucosidase, etc. carry out its degradation processes by breaking glycosidic bonds between glucose monomers of starch. Unripe fruits contain organic acids and these acids are stored in cell vacuoles. These acids are utilized in the tricarboxylic acid (TCA) cycle and glyoxylate cycle for the respiration processes. In most ripen fruits, there is a decrease in acidity due to TCA utilization in the respiratory process (Boggio *et al*., 2000). The cell wall degradation and softening of fruit during ripening take place with the action of various enzymes such as pectinases, cellulases, hemicelluloses and other texture-softening enzymes such as e-glycosidases, α-galactosidase, α-glucosidase, β-glucosidase, α-mannosidase, β-mannosidase, α-xylosidase, and β-xylosidase.

Presently, Sri Lanka depends on imported fossil fuel to fulfil the transportation fuel requirement. The possibility of producing first-generation bioethanol in Sri Lanka is limited due to the unavailability of extra agricultural lands. However, it has been estimated that approximately 270000 tons of the harvested fruits in Sri Lanka are wasted annually due to the improper post-harvesting activities such as improper packing, handling, transportation and storage throughout the marketing chain starting from the farmer to collector, whole seller, retailer and finally to the consumer (Kodippili, 2016). About 30% of banana, 46% of papaya, 18% of pineapple, 40 of lime, 41% of avocado and 90% of jackfruit are wasted from the annual production in Sri Lanka (Sarananda, 2000; Perera *et al*., 2009). Thus, it is a timely requirement to study the possibility of producing anhydrous bioethanol in Sri Lanka using these fruit wastes, as a gasoline substitute. It would minimize the dependence on imported fossil fuel, environmental pollution, solid waste management in the country, etc. (Borah & Mishra, 2011).

Microorganisms are used for the fermentation of substrates in the bioethanol production. Microorganisms employ several fermentation characteristics such as rapid and relevant carbohydrate fermentation ability, appropriate flocculation and sedimentation characteristics, genetic stability, ethanol tolerance, elevated concentrations of ethanol production, high cell viability for repeated recycling, and temperature tolerance (Doran, 2012). Out of many available microorganisms, yeast and bacteria are the most proven microorganisms for the production of ethanol through fermentation. Yeast species, *Saccharomyces cerevisiae* (commonly known as Baker's yeast) in *Saccharomyces* spp variety, are the more commonly used microorganism (Casey & Ingledew, 1986). PM, a novel microorganism (Gen Bank number of KU056820.1) has been identified recently as an efficient microorganism to produce bioethanol with fermentation. (Hewawasam *et al*., 2019). Presently, many varieties of genetically modified strains are used worldwide to produce bioethanol through fermentation. During the process of bacterial fermentation, the growth of bacterial cells takes place. This involves various biochemical networks and chemical reactions. Bioethanol yield depends on the proper manipulation of fermentation factors such as agitation to improve nutrient transfer to the cell surface, aeration to improve cell growth, temperature, pH, etc. (Lee, 2008). As such, it is essential to have prior knowledge of the kinetic behaviour of the fermentation process along with kinetic models before the large-scale bioethanol production (Farah *et al*., 2011; Ogbebor *et al*., 2014; Nanthaporn, & Niron, 2013).

Accordingly, this research aims at optimizing the kinetic modelling of bioethanol production by batch fermentation of selected three types of overripe fruits.

Fermentation is carried out at three different substrate concentrations, temperatures, and pH values for the optimization of bioethanol production. Optimization and kinetic modelling are carried to predict kinetic parameters and thus to select the best fermentation factors. This has been done by considering the highest correlation between the theoretical and actual curves by using the Monod model for glucose utilization and modified Gompertz equation for bioethanol production (Tussanee *et al*., 2015).

MATERIALS AND METHODS

Selection of fermentation parameters

Banana, papaya and jackfruits were used as substrates. Baker's yeast (*Saccharomyces cerevisiae*) and PM were used as inocula. Three different fermentation temperatures: 27, 32, and 35 oC, three different pH values: 4.3, 5.0, and 5.7 and three different concentrations of the fruit mass as 1:1, 1:1.5 and 1:2 (w:w) fruit to distil water ratio was selected. The settings of each variable were selected reference to the literature. (Hammond *et al*., 1996; Akin-Osaniye *et al*., 2008; Nirmal *et al*., 2012).

Separate fermentation experiments were carried out with all the possible combinations of fermentation parameters shown in Table 1.

Table 1: Experimental scenarios of the conducted experiments.

Fruit	Microorganism	Temperature / oC	pH	Fruit to water concentration/ (w:w)
Banana- embul kesel variety	*Yeast*	27	4.3	1:1
Papaya	*Yeast*	32	5.0	1:1.5
Jackfruit	*Yeast*	35	5.7	1:2
Banana- embul kesel variety	PM	27	4.3	1:1
Papaya	PM	32	5.0	1:1.5
Jackfruit	PM	35	5.7	1:2

Reagents and materials

Yeast extracts, peptone, and dextrose were purchased from Techno Pharm Chem (Delhi, India). Glucose was purchased from the local market. Agar, ammonium sulphate, magnesium sulphate heptahydrate were purchased from Marine Chemicals (India) and potassium dihydrogen phosphate was obtained from Loba Chemie Pvt Ltd (India). Potassium iodide and potassium dichromate were purchased from BDH Limited (England). Concentrated sulphuric acid and Sodium thiosulfate were purchased from Dae-Jung Chemical & Metal Co., Ltd, Korea and British Drug Houses Ltd. (London, UK) respectively.

Media for growing microorganisms

Yeast Extract–Peptone–Dextrose **(**YPD) media and glucose media were used to grow *Saccharomyces cerevisiae* and PM microorganism, respectively (Hewawasam *et al*., 2019).

Experimental procedure

Separate fruit juice samples with different solid contents were prepared by blending fruits and distilled water according to the required proportion in a domestic blender (Panasonic mixer grinder, model number – MX-AC300). 100.00 mL of each juice was then added in to sterile 250 mL conical flasks. The pH of each sample was set according to the sample number using a pH meter (HACH, Model No. 2786). The openings of the conical flasks were covered with cotton wool and aluminium foil for autoclaving. All the samples were then autoclaved for 30 min at 121 °C and allowed to cool up to room temperature. Experiments were carried out in duplicate.

Fermentation

The autoclaved fruit samples were used for fermentation. 1.00 mL of bacterial suspension (0.5 McFarland) was inoculated in to the autoclaved fruit medium inside a laminar flow. The conical flasks were then air tightened with sterile cotton wool and aluminium foil to ensure the anaerobic condition and then incubated (THOMAS AT 12R) at specified temperatures (27, 32, 35 oC) by shaking at 85 rpm for about 96 h with *Saccharomyces cerevisiae* and 144 h with PM microorganism for fermentation. 5.00 mL samples were taken out from each conical flask at 24-h interval, and the pH of the remaining fruit juice was adjusted to the specified level (HACH, Model No. 2786). The collected samples were centrifuged (TOMY, Model No. suprema 21) under 10000 rpm at -4 oC for 20 min to separate solid particles. The filtrate was collected to analyze the amount of reduction of sugar and ethanol concentrations. Finally, a confirmation run was carried out in triplicate by fermentation of banana fruit using PM microorganism under the selected best fermentation parameters.

Measurement of sugar and ethanol concentrations

The concentration of sugar in each filtrate was measured at 24-h time intervals using a calibrated refractometer (ATAGO, Model No.-1T) with a scale ranging from 0 to 30 % Brix unit. Ethanol concentrations of the filtrates were measured using Gas Chromatography (GC) (SHIMADZU, Model No. C 114850). The measured ethanol concentrations using the GC were verified randomly by the standard dichromate reagent method (William & Reese, 1950).

Statistical analysis

Kinetic model prediction, simulation, estimation of kinetic parameters, and correlations were carried out in MATLAB version R 2015a software.

Prediction of kinetic parameters

Prediction of the kinetic parameters was carried out by fitting the experimental data into empirical equations. Fermentation kinetic parameters such as maximum specific growth rate (μmax), half-saturation concentration (K_s), maximum ethanol concentration (P_m), maximum ethanol production rate (r_{pm}), and lag phase (t_L) were predicted reference to the theoretical graph relevant to the highest correlation between the experimental and theoretical graphs.

Monod equation (equation 1) was used to predict μmax and K_s (Tussanee *et al*., 2015).

$$\mu = \frac{\mu_{max} \cdot S_e}{K_S + S_e} \quad (1)$$

Where μ is the specific growth rate per h, and S_e is the substrate concentration in the effluent in g/L.

Modified Gompertz model (equation 2) was carried used to predict the kinetic parameters; P_m., r_{pm} and t_L (Tussanee *et al*., 2015).

$$P = P_m \exp\{-\exp\left[\frac{r_{p,m} \cdot \exp(1)}{P_m}\right] \cdot (t_L - t) + 1\} \quad (2)$$

Where, P is the ethanol concentration in g/L, and t is the fermentation time in h.

Theoretical equations

$$\text{Sugar utilization}/(\%) = \frac{\text{Amount of original sugar} - \text{Amount of residual sugar}}{\text{Amount of original sugar}} \text{ X } 100 \quad (3)$$

$$\text{Ethanol yield}/(\text{g-ethanol}/\text{g-gluecose}) = \frac{\text{Maximum ethanol concentration (g/L)}}{\text{Utilized glucose(g/L)}} \quad (4)$$

$$\text{Ethanol productivity}/(\text{g}/\text{L.h}) = \frac{\text{Maximum ethanol concentration (g/L)}}{\text{Fermentation time (h)}} \quad (5)$$

$$\text{Fermentation efficiency}/(\%) = \frac{\text{Actual ethanol yield (g/L)}}{\text{Theoritical ethanol yield(g/L)}} \text{ x } 100 \quad (6)$$

Data

Sugar concentration and ethanol concentration of the filtrates at 24-h intervals were measured.

RESULTS AND DISCUSSION

The highest R2 values of each fruit type and microorganism are observed and tabulated in Table 2 and Table 3 respectively.

Table 2: Sugar concentrations at 24-h time intervals.

Source	Microorganism	Sugar concentration(g/L)						
		0 h	24 h	48 h	72 h	96 h	120 h	148 h
Banana	Yeast	92.6	32.6	24.6	19.5	11.6		
	PM	87.6	43.4	34.0	20.3	19.5	14.5	10.1
Papaya	Yeast	50.7	26.1	19.5	12.5	10.9		
	PM	46.3	36.2	25.3	21.7	14.5	12.3	10.9
Jackfruit	Yeast	57.9	28.9	19.5	12.3	10.1		
	PM	65.1	39.8	32.6	21.7	12.3	10.9	10.1

Table 3: Ethanol concentrations at 24 h time intervals.

Source	Microorganism	Ethanol concentration / (g/L)						
		0 h	24 h	48 h	72 h	96 h	120 h	148 h
Banana	Yeast	0	2.3	6.2	10.3	5.3		
	PM	0	0.3	5.4	5.2	5.6	13.0	6.9
Papaya	Yeast	0	1.5	3.4	5.0	3.7		
	PM	0	0.4	3.0	2.7	2.9	5.3	3.2
Jackfruit	Yeast	0	2.1	4.3	6.8	4.6		
	PM	0	0.5	4.6	4.3	4.7	7.4	4.5

Analytical results

The calculated results on sugar utilization, ethanol yield, ethanol productivity, and fermentation efficiency for the three types of fruits using equations 3, 4, 5, and 6 are tabulated in Table 4.

Table 4: Fermentation and bioethanol production results.

Source	Microorganism	Sugar utilization/ (%)	Max. percentage ethanol concentration/ (g/L)		Fermentation time / (h)	Ethanol productivity/ (g/L.h)	Ethanol yield (g-ethanol/ g- glucose)	Fermentation efficiency/ (%)
			Actual	Theoretical				
Banana	Yeast	87	10.3	11.1	96	0.107	0.127	92.5
	PM	88	13.0	15.9	144	0.090	0.168	81.8
Papaya	Yeast	79	5.0	5.2	96	0.052	0.126	96.0
	PM	76	5.3	6.0	144	0.037	0.150	88.3
Jackfruit	Yeast	83	6.8	6.9	96	0.071	0.142	97.4
	PM	84	7.4	7.5	144	0.051	0.135	98.1

The data in Table 4 verifies that a higher percentage of sugar of all three types of fruits, ranging from 76 to 88%, has been utilized for bioethanol production. Minimum percentage of sugar utilization was observed from papaya fruit when ferment with PM microorganism. The maximum percentage of sugar utilization of 88% was observed with banana fruit when ferment with PM. The relatively higher percentage of sugar utilization by each fruit type is due to the higher ethanol tolerance of PM microorganism. Experimental and theoretical bioethanol yield for all types of fruits is relatively close, and actual ethanol yield is always lower than the theoretical bioethanol yield and the experimental results on ethanol concentrations are in an acceptable range comparative to the reported bioethanol concentrations by Akin-Osaniye et al., 2008, Suhas et al., 2013 and Brent *et al*., 1996. The actual and theoretical bioethanol yield of 13.0 g/L and 15.9 g/L, respectively, were observed using banana (embul variety) with PM which reveals that PM is a new collection to the bioethanol industry with a higher ethanol yield. From the confirmation run, carried out by fermentation banana fruit with PM at 32 oC temperature and 5 pH by maintaining fruit to water concentration of 1:1, a maximum ethanol yield of around 13.0 g/L was obtained. This amount of bioethanol yield is considerably high using a natural microorganism. Reference to the same table, ethanol yield of jackfruit with yeast is about 7.4 g/L, which is increased up to 7.5 g/L with PM. A study by Akin-Osaniye *et al*., in 2008, reported that the ethanol yield of jackfruit is 5.4 g/L with same fermentation conditions with *Saccharomyces cerevisiae*, and it has been increased by 2 g/L with PM (Akin-Osaniye *et al*., 2008). Considering the fermentation time, for all types of fruits, fermentation with PM has resulted in a long fermentation time of 120 hours with increasing bioethanol yield. This suggests that PM is a highly ethanol tolerant microorganism. The highest ethanol productivity of 0.107 g/L.h was observed with banana fruit with yeast and the highest bioethanol yield in grams ethanol over grams glucose was observes as 0.168 for banana fruit with PM microorganism (Table 4). These results appear to consistent with the results published in the literature by Tussanee *et al*., in 2015. The observed fermentation efficiencies in Table 4 are in a considerably higher range of 81.8% to 98.1%, which proves the maximum bioethanol productivity during the fermentation processes.

Selection of optimum fermentation parameters

The optimum fermentation parameters were obtained by considering the highest correlation between the experimental graph and theoretical graphs for each type of fruit with two different microorganisms. Monod equation (Equation 1) was used to obtain the theoretical graph of the variation of sugar concentration of the substrate with time for each fruit variety with each microorganism for estimating optimum fermentation process parameters and relevant fermentation kinetic parameters. Modified Gompertz equation (Equation 2) was used to obtain theoretical graphs of the variation of ethanol concentration in the filtrate with time for each fruit variety with each microorganism for estimating the ethanol production kinetic parameters. Corresponding graphs for the highest correlation with the experimental data are shown in Figures 1 and 2 respectively.

The highest correlation coefficients for the fermentation and ethanol production are given in Table 5. The best fermentation process parameters such as temperature, pH, and concentration of the fruit pulp: fruit to water ratio (w:w) for each fruit type with two different microorganisms are given in the same table.

Fermentation and ethanol production kinetic parameters, obtained from Monod model and Modified Gompertz equation are given in Table 6. Reference to Tables 4 and 5, the highest ethanol yield of 15.9 g/L has been predicted for the fermentation of banana fruit with PM at 35 ^{o}C temperature, pH of 5, and fruit to water concentration of 1:1. The lowest fermentation temperature and pH of 32 oC and 4.3, respectively, have been predicted for papaya fruit with fruit to water concentration of 1:1.5, resulting in maximum ethanol yield of around 5.0 g/L.

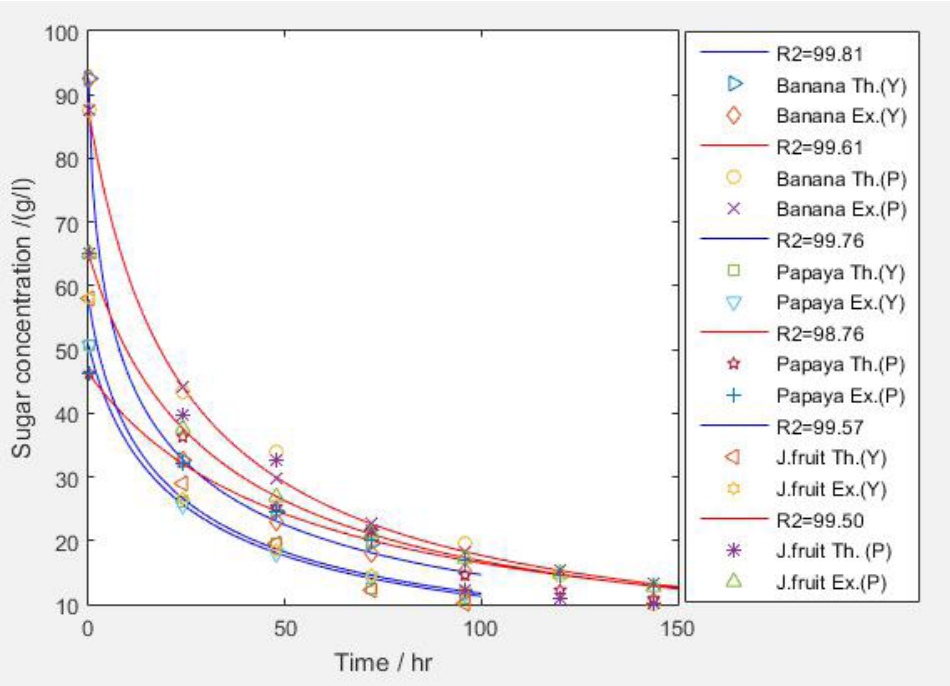

Figure 1: Variation of sugar concentration in the filtrate with time (considering the highest correlation of each fruit variety and each microorganism).

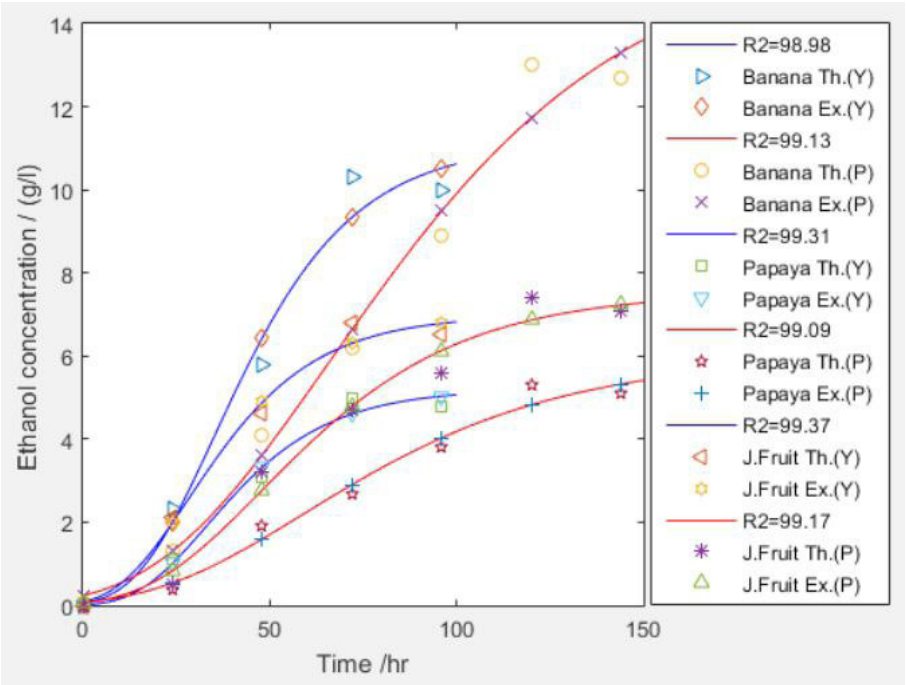

Figure 2: Variation of ethanol concentration the filtrate with time (considering the highest correlation of each fruit variety and each microorganism).

Table 5: Optimum fermentation parameters and correlations.

Fruit type	Microorganism	Temperature / ₀C	pH	Concentration (fruit:water) / (w:w)	Correlation (R_2)	
					Fermentation	Ethanol Production
Banana	Yeast	35	4.3	1:2	99.81	98.98
	PM	35	5.0	1:1	99.61	99.13
Papaya	Yeast	27	4.3	1:1.5	99.76	99.31
	PM	27	4.3	1:1.5	98.76	99.09
Jackfruit	Yeast	32	5.0	1:1.5	99.57	99.37
	PM	32	4.3	1:2	98.50	99.17

Table 6: Predicted kinetic parameters obtained from Monad and modified Gompertz models.

Source	Microorganism	Monad model		Modified Gompertz model		
		μmax /(g/g-VSS.h)	K_s/(g/L)	P_m/(g/L)	r_{pm}/(g/L.h)	t_L/(h)
Banana	Yeast	0.039	100	11.13	0.19	14.24
Banana	PM	0.309	720	15.90	0.13	20.24
Papaya	Yeast	0.067	120	5.21	0.11	14.62
Papaya	PM	0.246	656	6.00	0.06	19.22
Jackfruit	Yeast	0.052	100	6.98	0.13	9.21
Jackfruit	PM	0.135	340	7.54	0.09	17.56

The obtained best pH value of 4.5 for papayais in compatible with the data reported in the literature by Akin-Osaniye *et al*., in 2008. The best fermentation parameters for jackfruit using Yeast are 32 °C temperature, pH 5.0, and fruit to water concentration of 1:1.5. On the other hand, with PM microorganisms, the best fermentation parameters/ conditions of 32 oC temperature, pH of 4.3 and fruit to water concentration of 1:2 have been observed. The fermentation process parameters using yeast, tabulated in Table 5, are in compatible with the data reported by Akin-Osaniye *et al*., 2008. It was noticed that the bioethanol yield of all the fruits has been increased with PM microorganism.

Considering to the correlations in Table 5, all the correlations are in a higher range, above 98.50, which indicates that the bioethanol production process is well explained by the Monod equation and modified Gompertz equations. Besides, the received graphs in this study are quite similar to the graphs obtained by a previous study reported Tussanee *et al*., in 2015.

Prediction of kinetic parameters

The kinetic parameters in Table 6 were obtained by performing kinetic analysis with Monod and modified Gompertz equations in MATLAB by considering the graphs obtained with the highest correlations. Values for the maximum specific growth rate (μmax) and saturation concentration (K_s) were obtained from Monad equation while maximum ethanol concentration (Pm), maximum ethanol production rate (r_{pm}) and lag time (from the beginning of fermentation to exponential ethanol production) (t_L) were obtained using modified Gompertz equation. According to Table 5, the values for μmax were found in the range of 0.039 to 0.309. These values seem to be similar to the values, reported in the literature (Ariyanti & Hadiyanto, 2013; Farias *et al*., 2014). Usually half-saturation concentration, K_s is a low value but the results of $K_{s,}$ obtained in this study are relatively high due to higher substrate concentration, which is commonly found in ethanol production (Nadya *et al*., 2012). The calculated values for P_m for fermentation of the three fruits with yeast are in agreement with the values reported in previous studies on ethanol production (Nadya *et al*., 2012). Values for r_{pm} and t_L among the three different types of fruits studied were found in the range of 0.06-0.19 g/L.h and 9.21-20.24 h respectively and these values, validate the results of previous studies by Tussanee *et al*. in 2015. These kinetic parameters could be useful to design the bioreactor for commercial-scale ethanol production with these overripe fruit masses.

CONCLUSIONS

Fermentation of overripe banana, papaya and jackfruit was successfully carried out in batch fermentation and the fermentation results were used for the optimization and kinetic parameter estimation of the fermentation process. Banana was identified as the most potential freely available fruit in Sri Lanka for the bioethanol production using a novel microorganism, PM. The highest bioethanol yield of about 13.0 g/L was obtained by fermentation banana fruit mass with a concentration of fruit mass to water ratio of 1:1 using PM microorganism by fermentation at 35 oC temperature and pH of 5. The fermentation process was well explained by the Monod equation and modified Gompertz equations with high correlations of 99.61 and 99.13 respectively.

Considering the experimental bioethanol yield and

the available banana waste data of Sri Lanka in 2017, approximately $2x10_6$ litters of anhydrous bioethanol could be produced annually by utilising whole available banana waste in Sri Lanka. Further, the residual materials of the bioethanol production processes could be used as an eco-friendly, non-toxic and biodegradable fertilizer.

CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

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