Standardization of fermentation conditions for the production of bio-ethanol from matured coconut water

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Abstract. Pollution of natural, environmental and water resources caused through industrial wastes is becoming a major concern nowadays. Agro-industries contribute 10-15% for the cause of pollution. As the wastes generated from agro-industries are of biological origin, they can be effectively reused; if their alternate uses are explored. Coconut industry is one of the major agro-industry where wastes are generated in large quantum. Wastes generated in coconut industry include coconut shell, coconut husk, coconut water and oil cake. The unutilized coir industry waste includes coconut water which is generated during copra processing. Coconut waste water contains more amounts of sugars, lipids, organic acids, etc and hence they can be used as a feedstock for bio-ethanol production. In this study an attempt was made to standardize fermentation conditions for enhanced bio-ethanol production from matured coconut water. Standardized fermentation conditions for enhanced bio-ethanol production were: temperature-37.5ºC, pH-5.5 and incubation period-96 hours with Saccharomyces cerevisiae. From our study it was inferred that matured coconut water is a potential source for production of bio-ethanol and this will lead to circular economy in coconut processing industry.

Keywords: Matured coconut water, bio-ethanol, fermentation.

1 Introduction

Coconut is one of the primary plantation crops grown in India, covering approximately 1975 hectares under cultivation. Tropical Asian nations lead in global coconut output, with Indonesia, Philippines and India accounting for more than 72% of total production. India ranks third with an impressive nut harvest of 21,665

million nuts from 81 thousand hectares, according to recent research. Additionally, coconut is renowned for its wide array of by-products including coconut milk, virgin coconut oil, coconut chips and more.Studies indicate that, matured coconut water due to its rich nutritional properties could be used as a potential feedstock for bioethanol generation. Industrial mechanization is a boon to the developing nations with the advantages such as reduction in labour costs and increased productivity. Increased number of industries and mechanization has resulted in increased waste disposal into the

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environment. Agro-industries contribute to waste disposal to a considerable level and this waste management costs huge to the respective industries. Conversion of these biodegradable wastes into biofuels and value-added chemicals is gaining momentum as it holds two main advantages such as additional revenue and effective waste management. Copra industry is one of the major coconut based agro industry, in which a large volume of coconut water is generated and discarded into the environment. This leads to changes in soil properties, groundwater leaching problems and emission of foul smell due to fermentation. Coconut water is nutritionally rich and hence this can be used as a raw material for production of biogas and bio-ethanol. Whilst cane molasses is the usual preferred raw material, the supply however had been recorded to become more exhausted due to the rise of fermentation industries. Furthermore, this had also affected the increase in the molasses price in recent years. Therefore, in the effort to employ homeland resources, overly matured coconut water (MCW), which often regarded as waste, seems to be an attractive renewable carbon source for ethanol fermentation industry. Hence it is proposed that utilization of MCWfor ethanol production will be advantageous as theMCW may support fermentative yeast growth and ethanol production

2 MATERIALS AND METHODS

2.1 Physiochemical analysis of MCW

Samples of Matured coconut water were collected from 10 copra industries located in Tiruppur districts. Matured coconut water was filtered through

muslin cloth used for analysis

2.1.1 Total acidity

Total acidity was determined by titrimetric method. Standard procedure given by International Science Congress Association was followed. Ten different samples of matured coconut water were taken in a conical flask with phenolphthalein as indicator. The solution was titrated against 0.1N NaOH solution. The progress of colour change was observed and calculated with

$$Total acidity = \frac{Titrate Value \times 1000}{Volume Of Samples Taken} \tag{1}$$

The total acidity is expressed in terms of CaCO3 mg/l

2.1.2 Total lipids

Total lipids estimation was done by KOH method. Ten samples of matured coconut water were taken with phenolphthalein as indicator and titrated against 0.1N potassium hydroxide. Total lipids were expressed in terms of mg/l

$$Total lipids = \frac{titrate value * normality of KOH * 40}{weight of the sample} \tag{2}$$

2.1.3 Dissolved oxygen

Dissolved oxygen was assessed by modified wrinkler's method. Ten samples of matured coconut water were taken in BOD bottles with 2ml of magnesium sulphate, alkali azide, and add 2ml of concentrated sulfuric acid. The mixture was titrated against sodium thiosulphate with starch as indicator. The dissolved oxygen was calculated with the formula

$$Dissolvedoxygen = \frac{V * N * 8 * 1000}{Weightofthesample} \tag{3}$$

Dissolved oxygen was expressed in terms of mg/l

2.1.4 Total suspended solid (TSS)

Total suspended solids were analysed with gravimetric method. Ten different samples of matured coconut water were taken and filtered through the Whatman No.1 filter paper. The filter paper was oven dried at 65oC for 5hrs. As the drying progressed the filter paper was weighed to know the final weight. The suspended solids were expressed in mg/l and calculated by

$$TSS = \frac{w2}{w1} \times \frac{1000}{v} \tag{4}$$

Where W1 – initial weight, W2 – final weight, V - volume of the sample

2.1.5 Total dissolved solids (TDS)

Total dissolved solids were determined by gravimetric method. Ten filtered samples of matured coconut water was taken in a porcelain dish and kept in the oven overnight at 105oC. As the drying progresses the weight was determined and calculated by

$$Formula = \frac{w2}{w1} \times \frac{1000}{v} \tag{5}$$

Where W1 – initial weight of the porcelain dish, W2 – final weight of the porcelain dish, V – volume of the sample.TDS was expressed in mg/l.

2.2 Total solids (TS)

Total solids were determined by the addition of total suspended solids and total dissolved solids

$$TS = TSS + TDS \tag{6}$$

2.2.1 Total sugars

The total sugar content of MCW was calculated using FSSAI method. It is calculated by multiplying the reducing sugar percentage by 0.95. The total sugar content was expressed in percentage (%). Total sugar = Reducing sugar percent x 0.9

2.2.2 Reducing sugar

Reducing sugar are fermentable sugars. Ten samples of matured coconut water were taken in test tube containing 1ml of DNS reagent and kept in water bath for 20mins. As the progress in colour change occurs the spectrum was analyzed in spectrometer at 540nm. The concentration was obtained by plotting the unknow concentration against standard concentration.

2.2.3 Total proteins

Total proteins were assessed by biuret method. Biuret reagent was prepared by dissolving 1.5g of copper sulphate in 375ml of 2M sodium hydroxide, the volume was made up to 1litre. In a fresh clean test tube 0.4, 0.2ml of MCW was taken separately and 1ml of biuret reagent was added. Standard albumin solution was prepared and volume of 0.2, 0.4, 0.6, 0.8 and 1 ml was taken and added with one ml of biuret reagent. The solution was examined under UV spectrophotometer at 540nm. The concentration of protein was obtained by plotting the values in graph and expressed in mg/l.

2.2.4 NPK content

MCW samples were analysed for total NPK content by Kjeldahl method for nitrogen, spectrophotometric method for phosporous, flamephotometric method for potassium. For total nitrogen, 1 to 2ml of sample was taken in 500ml of Kjeldahl flask and 0.7mg of mercuric oxide, 15 of potassium sulphate, and 40ml of concentrated sulfuric acid were added and kept for digestion overnight. The digested sample was taken in a conical flask and titrated against 0.1N sodium hydroxide with methylene blue as indicator. For total phosphorous, 1-2ml of sample was taken and 0.45ml of H2SO4and 3.9ml of deionized water was added. About 0.5ml of ammonium molybdate and 0.5ml of ascorbic acid was added and the test tube was closed with screw cap. The mixture was heated to 100oC for 7mins and allowed to cool. The samples were analysed by spectrophotometer at 820nm. For total potassium, 1ml of MCW sample was taken in 100ml of conical flask and added with 15 ml of triple acid mixture. The contents were digested till the colour disappears in sand bath. After digestion, the samples were made up of 100ml with distilled water. The samples were analysed by flame photometer.

2.3 Inoculum preparation

Saccharomyces cerevisiae available in the Department of Renewable Energy Engineering was used to conduct the fermentation studies. The yeast culture was streaked in Yeast extract Peptone Dextrose (YPD) agar (glucose 2%, peptone 2%, yeast extract 1%). After 24hrs of incubation single colonies were taken and incubated in fresh YPD broth at room temperature. After incubation for a clock service, the cells were centrifuged at 6000rpm for 10 min. then diluted in sterile distilled water and used for fermentation studies.

2.4 Fermentation conditions

Batch fermentation was carried out in 250 ml conical flask with 100 ml of fermentation medium. The Fermentation medium consists of filtered Matured Coconut Water inoculated with 1% yeast inoculum and incubated at different temperatures and pH. To determine the fermentation duration, reducing sugar levels was analysed every 24 hours by Di-Nitro Salicylic acid (DNS) method.

2.5 Optimization conditions

Experiments were designed using a Central Composite Design to study the different levels of two independent parameters.For two parameters, the model equation is

$$Y = \beta_0 + \beta_{1\times1} + \beta_{2\times2} + \beta_{11\times12} + \beta_{22\times22} + \beta_{12\times1\times2} \tag{7}$$

Table 1: Central composite design levels

Variables	Units	$-\alpha$	-1	0	$+1$	$+\alpha$
X 1: Temperature	Celcius	26.9	30	37.5	45	48.1
X 2:PH	-	4	4.5	5.5	6.5	6.9

*TSS-Total Suspended Solids; TDS-Total Dissolved Solids & TS-Total Solids

2.5.1 Ethanol quantification

The concentrations of bio-ethanol were determined using HPLC (Shimadzu), with BIO-RAD column (HPX-87H), a refractive index (RI) detector. The mobile phase utilized was H2SO4 with a concentration of 0.065mM flowing ata rate of 0.6 ml/min. A 20 μL injection volume was used for all samples. The concentration was determined by comparison with standards (1000ppm ethanol standard).

3 RESULTS AND DISCUSSION

Matured coconut water, a major industrial effluent from copra industry (Tanqueco et al, 2007), a nutrient-rich source is discarded into the environment as waste. This can be potentially used as a feedstock for production of bioethanol and this study was an attempt to standardize fermentation conditions for enhanced bio-ethanol production from matured coconut water and the results were presented below. Ten samples collected from copra industry were analysed for physiochemical properties. The results indicated the relatively high levels nutrient composition of MCW as follows: total potassium at 1072 mg/L, total solids at 664 mg/L, total acidity at 45. mg/L, total sugar at 1. 65%, total nitrogen at 0.12%, total phosphorus at 10. 8 mg/L, total lipids at 0.0936mg/l and dissolved oxygen at 23 mg/L.

Table 2: Compositional analysis of Matured Coconut Water – TSS, TDS & TS

MCW Sample	TSS (mg/l)	TDS (mg/l)	TS (mg/l)
S1	1.8	813.0	814.8
S2	3.6	638.5	642.1
S3	3.1	506.4	509.5
S4	3.1	491.7	494.8
S5	2.6	611.6	614.2
S6	2.1	855.5	857.6
S7	4.9	486.2	491.1
S8	2.9	880.5	883.4
S9	5.5	738.7	744.2
S10	4.3	591.8	596.1
Average	3.4	661.4	664.9

Statistical Design Expert version 6.0.7 was used for design of experiments and graphical analysis of data. The design expert generated a total of 13 sets of fermentation trials.

Table 3: Compositional analysis of matured coconut water - total acidity, total lipids & dissolved oxygen

MCW Sample	Acidity(mg/l) as CaCO 3	Total Lipids (mg/l)	Dissolved Oxygen(mg/l)
S1	50	0.16	28.4
S2	58	0.08	28.0
S3	60	0.08	21..6
S4	30	0.07	32.8
S5	40	0.08	22.4
S6	46	0.07	19.6
S7	42	0.08	23.2
S8	40	0.07	20.8
S9	50	0.16	18.4
S10	38	0.08	20.0
Average	45.4	0.09	23.5

Fermentation duration was determined by the decreasing concentration of reducing sugars. Initially, the reducing sugar concentration was about 7.5g/l after 24hrs it was reduced to 5.4g/l and the final concentration was 2.3g/l at 90hrs. Spectrophotometric analysis for the determination of reducing sugar concentration is a very sensitive, low- cost, easy to handle, and accurate method. The present study demonstrated that the initial reducing sugar concentration gradually decreases during fermentation during bioethanol fermentation due to utilization of reducing sugars for bioethanol production.

Table 4: compositional analysis of matured coconut water- total sugar, reducing sugars & total protein

MCW Sample	Total Sugar %	Reducing Sugar (g/l)	Total Protein(mg/l)
S1	1.93	7.5	0.001
S2	2.04	7.0	0.001
S3	1.96	7.6	0.012
S4	1.91	6.2	0.001
S5	1.94	7.3	0.007
S6	1.93	7.9	0.001
S7	2.01	7.5	0.001
S8	2.00	6.0	0.001
S9	1.95	7.5	0.002
S10	1.98	6.9	0.001
Average	1.97	7.5	0.003

In this optimization study, Saccharomyces cerevisiae was used to carry out fermentation trials. Fermentation trials were designed by design expert, with a total of 13 sets of two independent factors. The coefficient of Response Surface Model was evaluated by regression analysis and tested for their significance. The coefficient of determination (R2) of the model is 0.9369. This makes the model more suitable for the relationship among the parameters.The summary of regression values was tabulated in table.7 showed the actual and predicted ethanol yield.

The 13 sets of fermentation trials were generated (table.4) and we can compare the difference between the actual and RSM predicted yield (Fig.1& 2 represent the comparison between two yields). The optimal condition for bioethanol production from matured coconut wastewater was determined to be 37.5 $^{\circ}C$ and 5.5 pH. S.cerevisiae rapidly utilized 50% of the fermentable sugar at this optimum temperature (Zoppellari & Bardi, 2013). The optimization study results using RSM software suggests $-\alpha$ and $+\alpha$ value such a way of low pH and low temperature. However, the suggested pH of 4 and temperature of 26.5 degree celcius yields the lower ethanol. Singh and Bishnoi, (2012) studied the bioethanol production from alkali rice straw and reported that an increase in the pH decreases the ethanol yield and the same trend was observed in our study i.e., when the pH is increased from 4.5 to 5.5 the ethanol yield decreased. Findings of our study is in accordance with Peng and Chen, (2011) who reported that with increase in temperature beyond the optimum level the ethanol yield will be reduced. Lin et al., (2011). reported that high temperatures reduced the ethanol production due to reasons such as inhibition of yeast cell growth, impairment in transportation of nutrients into the cell and toxic compound accumulation. The optimum temperature range for Saccharomyces cerevisiae growth and ethanol fermentation is reported to be between 28°C to 38°C; temperatures above this threshold may hinder yeast cell proliferation and metabolic activity. Underanaerobic conditions, S. cerevisiae converts glucose into ethanol and carbon dioxide without using oxygen. This fermentation pathway is less thermodynamically favourable than aerobic respiration, yet it allows the yeast to survive in low-oxygen environments. Research shows the optimal temperature for anaer-

Table 5: Compositional analysis of matured coconut water – NPK content

MCW Sample	Total Nitrogen(%)	Total Phosphorous(mg/l)	Total Potassium(mg/l)
S1	0.175	10.98	1521.0
S2	0.124	10.65	1035.0
S3	0.116	11.43	533.8
S4	0.102	10.46	853.9
S5	0.113	10.56	1154.0
S6	0.156	10.80	1067.0
S7	0.143	11.02	1267.0
S8	0.126	10.78	1156.0
S9	0.128	10.38	1044.0
S10	0.138	11.89	1097.0
Average	0.132	10.90	1072.8

Table 6: Reducing sugar concentration

Time	OD Value	Reducing Sugar (g/l)
0hrs	0.02385	7.5
24hrs	0.01883	5.4
48hrs	0.01418	3.9
72hrs	0.0123	3.25
96hrs	0.0089	2.3

obic respiration in S. cerevisiae to be approximately 35°C; temperatures above or below this point can influence fermentation performance and strain population dynamics.[1][2][3][4][5][6][7][8][**?**]

4 Conclusion

Agro industries are one of the major contributors for environmental pollution and accounts for about 10-15% for pollution. The major advantage of wastes generated from agro industries is that they can be effectively reused and recycled. Nowadays coconut based agro industries is gaining importance as because value added products from coconut is increasing day by day. In parallel the waste generated from coconut based agro industries is also increasing in a considerable level, which needs to be addressed at this juncture to reduce the level the pollution. The matured coconut water contains an affordable amount of contents such as Total Suspended Solids (TSS), Total Dissolved Solids (TDS), total nitrogen, phosphorus, potassium, total sugar, reducing sugars and total lipids for bioethanol production. The results found relatively high levels of total potassium at 1072 mg/L, total solids at 664 mg/L, total acidity at 45. mg/L, total sugar at 1. 65%, total nitrogen at 0.12%,

Table 7: Summary of regression values

Values	Ethanol fermentation
Std. Dev.	0.1112
Mean	2.50
C.V.R2	0.9639
Adj R2	0.9381
Pred R2	0.7433
Adeq precision	16.0269

Table 8: Optimization of fermentation trials

X 1:Temprature	X 2:PH	Actual Yield	RSM predicted
37.5	6.9	2.40	2.55
37.5	5.5	2.95	2.95
37.5	5.5	2.95	2.95
30.0	4.5	2.29	2.32
45	4.5	2.25	2.17
45	6.5	2.31	2.13
30	6.5	2.31	2.23
48.1	5.5	1.60	1.74
37.5	5.5	2.95	2.95
37.5	4.0	2.65	2.65
26.9	5.5	1.91	1.91
37.5	5.5	2.95	2.95
37.5	5.5	2.95	2.95

total phosphorus at 10. 8 mg/L, total lipids at 0.0936mg/l and dissolved oxygen at 23 mg/L. And this study it was evident that, matured coconut water can be potentially used as a raw material for production of bio-ethanol. Favourable conditions for bioethanol production from MCW were found to be 37.5oC and 5.5 pH. Since the yield of bio-ethanol is less when compared to cane molasses, future research can be done on supplementation with nutritionally rich substitutes like fruit wastes, lignocellulosic biomass, starchy residues, etc., and this will pave way to enhance the bio-ethanol production from MCW.

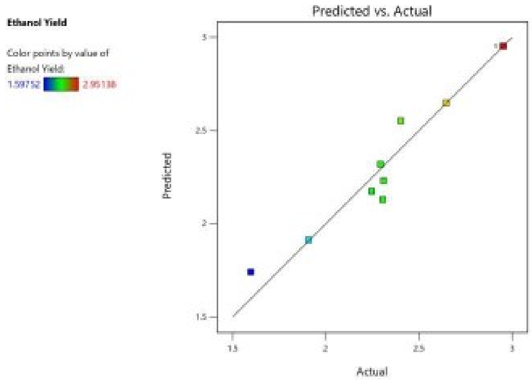

Fig. 1. Comparison between two yields

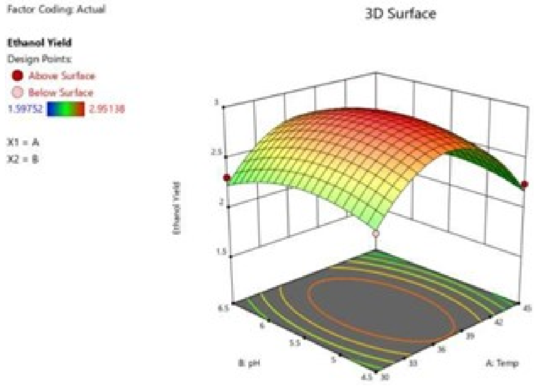

Fig. 2. 3D representation of actual yield and predicted yield

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