

## Response of Machine-Driven Ethanol Yield on the Production Input Ratio of Inoculum Size and Microbial Growth

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**Abstract:** The response of machine-driven ethanol yield to the production input ratio of inoculum size and microbial growth has been evaluated. The response analysis was carried out within a range of process parameters such ethanol yield, inoculum size, microbial growth and input ratio IS/ MG; 4.99-11.63 (%), 1-3 (O.D), 0.98-1.79 (O.D) and 1.0204 – 1.6854 respectively. An empirical model derived to assess the response of the ethanol production to the synergistic influence of the inoculum size and microbial growth as an input ratio IS/MG indicates that ethanol yield is almost a quadratic function of the input ratio IS/MG. Model generated results indicate that ethanol yield decreases with increased IS/MG ratio in line with experimental result until at a certain value of IS/MG where the ethanol yield drops. Differentiation of the model shows that ethanol production is minimum (1.8028 %) at IS/MG of 5.6092 above which the yield rises. The model;  $\zeta \approx 11.7(\theta/\gamma)^{-1.65} + 0.2(\theta/\gamma) + 0.001$  predicts the ethanol yield with maximum deviation < 7.5% (from actual results). This translated into over 92.5% operational confidence levels for the derived model. The validity of the model was rooted on the core model expression  $\zeta - S \approx N(\theta/\gamma)^{-K} + H(\theta/\gamma)$  where both sides of the expression are correspondingly almost equal. The standard error incurred in predicting the ethanol yield relative to values of the actual results is 0.16%. The correlation coefficients between ethanol yield and inoculum size, microbial growth and IS/MG ratio were all > 0.88.

**Key words:** Machine - Driven ethanol yield - Input ratio of inoculum size - Microbial growth

### INTRODUCTION

Diversification in energy generation stems on the need and demand for cheap fuel usable in transportation and machine-driving in industries. Ethanol (99% pure alcohol by volume) is used as fuel, having been produced from agricultural products such as cassava and molasses through fermentation process [1]. The consideration of alternative energy production in the form of ethanol from agricultural products and wastes was anchored on the limitations of fossil based energy yield which goes with environmental and human health hazards. Ethanol is a biofuel in nature with renewable sources, and produced with quasi-zero CO<sub>2</sub> [2].

All over the world, governments have encouraged various kinds of biofuel production based on the looming energy crisis that appears to be far from any

possible remedy. The higher price of oil has attracted the greater attention to biofuels, especially bioethanol, biodiesel, biohydrogen etc.

Research [3] has shown that biofuel is classified based on its sources. The first generation biofuel were produced from carbohydrate, lipids, oils, agro-products and wastes using conventional technologies. The second generation biofuel are produced from lignocellulosic biomass including lignocellulosic plant biomass such as stems, wood etc. Biofuel from this generation includes biohydrogen, biomethanol and mixed alcohol, many of which are still under research and development.

Attention has been shifted to the use of new sources of vegetable fibers to replace wood raw materials for pulp and paper applications. Banana (*Musa acuminata*) has been found [4] to be suitable for

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such application due to its contents and socioeconomic importance in so many countries. In India where it is the most important fruit crop, it contributes 27% of world production.

Report [5] has listed regional production, harvesting processing factors, and recoverability factors as functionalities on which the potential of agriculture residues depends.

High transportation cost has been fingered as the major challenge in using agriculture residues such as cotton stalks, wheat and rice straw, coconut shells, maize cobs, jute sticks, and rice husks as the main fuel in power stations [6]. This makes the whole project very expensive.

Forestry waste which includes wood chips, sawdust, and bark can provide 65% of the biomass energy potential [7, 8]. Forestry residues include biomass, not harvested or removed from sorting regions in commercial hardwood and softwood production, through forest management operations such as pre-commercial thinning and removal of dead and dying trees.

So many researchers [9-11] have successfully produced ethanol from agro-wastes such as sweet sorghum bagasse, raw wheat straws and sugarcane bagasse. Results of fuel ethanol produced from sweet sorghum bagasse using microwave irradiation shows an ethanol yield based on total sugar of 480 g/ kg and marginal land at 0.252m<sup>3</sup>/ton biomass. Fermentation of biologically pretreated wheat straw for ethanol production [10] revealed that the highest overall ethanol yield was obtained with the yeast *Pachysoletanophilus*. The recorded yield was 163mg ethanol per gram of raw wheat straw (23 and 35% greater).

Results of investigation [11] on the ultrasonic-assisted enzymatic saccharification of sugarcane bagasse for bioethanol production indicated maximum glucose yield as 91.28% of the theoretical yield, while the maximum amount of glucose obtained was 38.4 g/L (MTCC 7450). Furthermore, the result revealed the percent hydrolyte obtained as 91.22% of the theoretical ethanol yield (MTCC 89). It was observed from the research that reaction time decreases. The investigation also revealed that application of low intensity ultrasound enhanced the enzyme release and intensified the enzyme-catalysed reaction.

Empirical models have been derived [12- 14] for predictive analysis of the ethanol yield relative to different input and output process parameters at various process conditions. The essence of the models was to evaluate the response of ethanol yield to the process parameter affecting it.

The present work aims at assessing the response of a machine-driven ethanol yield to the production input ratio of inoculums size and microbial growth. The results generated will be graphically compared with those generated from the experiment and the level of deviation determined.

## MATERIALS AND METHODS

A weighed quantity of prepared sugar cane molasses was put in a reaction vessel containing the appropriate *Saccharomyces cerevisiae*. Details of the experimental procedure and associated process conditions are as stated in the past report [1]. Analysis of the bio-ethanol production was carried out based on the input ratio of inoculums size and microbial growth using a derived and validated empirical model.

**Model Formulation:** Experimental data obtained from research work [1] were used for this work. Computational analysis of the data shown in Table 1, gave rise to Table 2 which indicate that;

$$\zeta - S \approx N(\vartheta/\gamma)^{-K} + H(\vartheta/\gamma) \quad (1)$$

Introducing the values of S, N, K and H into equation (5) reduces it to;

$$\zeta - 0.001 \approx 11.7(\vartheta/\gamma)^{-1.65} + 0.2(\vartheta/\gamma) \quad (2)$$

$$\zeta \approx 11.7(\vartheta/\gamma)^{-1.65} + 0.2(\vartheta/\gamma) + 0.001 \quad (3)$$

S = 0.001, N = 11.7, K = 1.65 and H = 0.2 are empirical constants (determined using C-NIKBRAN [15])

where,

- ( $\vartheta$ ) = Inoculum size, IS (O.D);
- ( $\gamma$ ) = Microbial growth, MG (O.D)
- ( $\zeta$ ) = Ethanol yield conc. (%);
- ( $\vartheta/\gamma$ ) = Input ratio IS/ MG

## RESULTS AND DISCUSSION

Table 1: Variation of ethanol yield concentration  $\zeta$ , with inoculums size  $\vartheta$ , microbial growth  $\gamma$  and input ratio ( $\vartheta/\gamma$ ) [1]

( $\vartheta$ )	( $\gamma$ )	( $\vartheta/\gamma$ )	( $\zeta$ )
1.0	0.980	1.0204	11.63
1.2	1.144	1.0490	11.31
1.5	1.390	1.0791	10.83
2.5	1.790	1.3966	7.02
2.8	1.782	1.5713	5.48
3.0	1.780	1.6854	4.99

**Boundary and Initial Condition:** Consider sugar cane molasses interacting with microbes. The atmosphere in the reaction vessel was not contaminated i.e (free of unwanted gases and dusts). Range of ethanol yield, inoculum size, microbial growth and input ratio IS/ MG used are 4.99- 1167 (%), 1- 3 (O.D), 0.98-1.79 (O.D) and 1.0204 – 1.6854 respectively. Furthermore, reaction time and treatment temperature used were maintained constant at 72hrs and 30°C respectively. Mass of wastes used and other process conditions are as stated in the experimental technique [1].

The prevailed boundary conditions are: anaerobic atmosphere to enhance microbial action on the sugar cane molasses. At the bottom of the particles, a zero gradient for the gas scalar are assumed and also for the gas phase at the top of the waste particles. The biodegraded waste was stationary. The sides of the waste particles are taken to be symmetries.

**Model Validity:**The validity of the model is strongly rooted on the core model equation (1) where both sides of the equation are correspondingly almost equal. Table 2 also agrees with equation (1) following the values of  $\zeta - S \approx N(\vartheta / \gamma)^{-k} + \int (\vartheta / \gamma)$  evaluated from the actual results in Table 1. Furthermore, the derived model was validated by comparing the ethanol yield predicted by the model and that obtained from the experiment. This was done using various analytical techniques which includes statistical, graphical, calculus and deviational analyses.

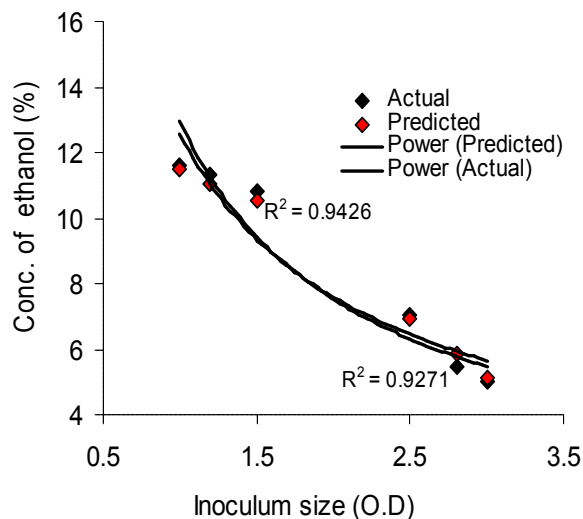


Fig.1: Coefficient of determination between ethanol yield and inoculums size as obtained from actual and model-predicted results

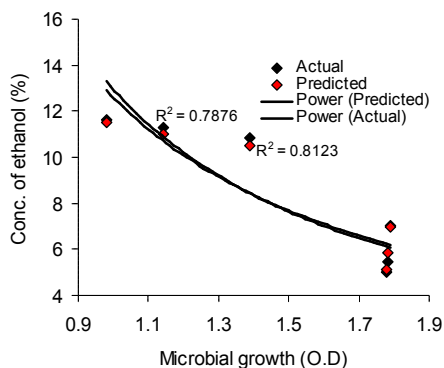


Fig.2: Coefficient of determination between ethanol yield and microbial growth as obtained from actual and model-predicted results

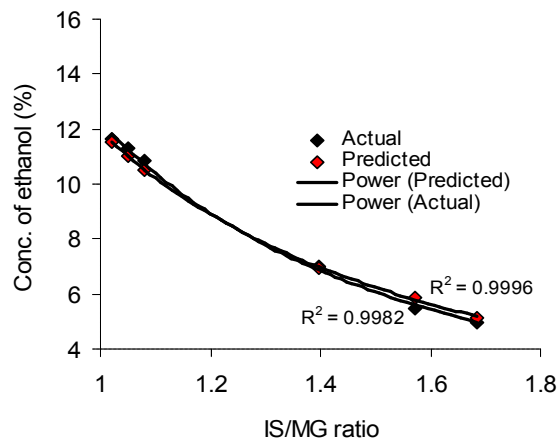


Fig.3: Coefficient of determination between ethanol yield and IS/ MG as obtained from actual and model-predicted results

**Statistical Analysis**

**Correlation:** The correlation coefficient between ethanol yield and inoculums size, microbial growth and IS/ MG were evaluated (using Microsoft Excel Version 2003) from results of the actual and derived model. These results are 0.9627 & 0.9709, 0.8875 & 0.9013 and 0.9991 & 0.9998 respectively. The evaluations were based on the coefficients of determination  $R^2$  shown in Figs. 1- 3 and then calculated using equation (4).

$$R = \sqrt{R^2} \tag{4}$$

**Standard Error (STEYX):** The standard error incurred in predicting the model-based ethanol yield relative to values of the actual results is 0.16%. The standard error was evaluated using Microsoft Excel version 2003.

**Graphical Analysis:** The validity of the derived model was further verified by plotting values of the actual, besides the model-predicted results using Microsoft Excel (version 2003) to evaluate the trend of both results. Figs. 4-6 indicate very close alignment of curves and shapes which depicted significantly similar trend of data point's distribution for the actual and derived model-predicted inhibition efficiency. This shows proximate agreement between both results.

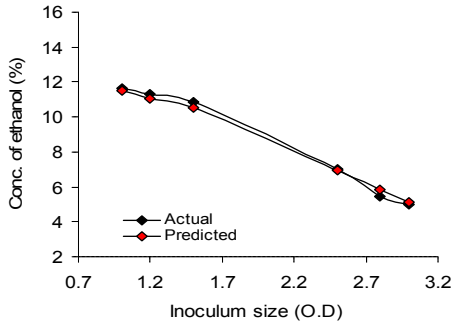


Fig. 4: Variation of ethanol yield with inoculums size as obtained from actual and model-predicted results

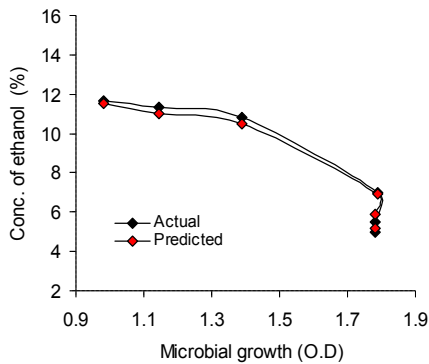


Fig. 5: Variation of ethanol yield with microbial growth as obtained from actual and model-predicted results

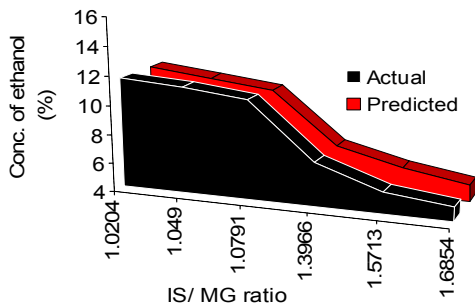


Fig. 6: Variation of ethanol yield with IS/ MG as obtained from actual and model-predicted results

**Predictive Evaluation of IS/MG at Minimum Ethanol Production:**

Recalling the derived model in equation (3)

$$\zeta \approx 11.7(\vartheta/\gamma)^{-1.65} + 0.2(\vartheta/\gamma) + 0.001;$$

Differentiating equation (3) with respect to  $(\vartheta/\gamma)$  and equating to zero;

$$0 = -19.305(\vartheta/\gamma)^{-2.65} + 0.2 \tag{5}$$

$$19.305(\vartheta/\gamma)^{-2.65} = 0.2 \tag{6}$$

$$(\vartheta/\gamma)^{-2.65} = 0.01036 \tag{7}$$

Taking Logarithm of both sides of equation (7);

$$\text{Log}(\vartheta/\gamma)^{-2.65} = \text{Log} 0.01036 \tag{8}$$

$$-2.65 \text{Log}(\vartheta/\gamma) = \text{Log} 0.01036 \tag{9}$$

$$-2.65 \text{Log}(\vartheta/\gamma) = -1.9846 \tag{10}$$

$$\text{Log}(\vartheta/\gamma) = 0.7489 \tag{11}$$

$$\vartheta/\gamma = 10^{0.7489} \tag{12}$$

$$\vartheta/\gamma = 5.6092$$

This is the optimal value of  $\vartheta/\gamma$  for which the ethanol yield is minimum.

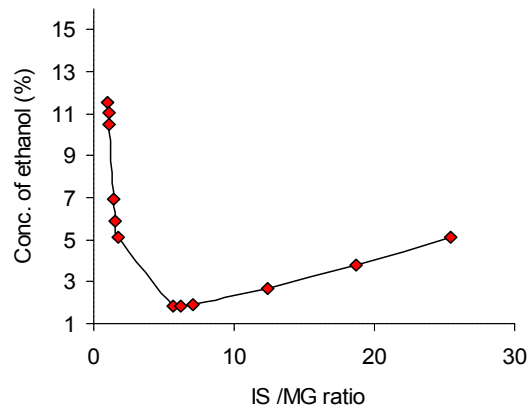


Fig. 7: Variation of model –predicted ethanol yield with IS/ MG ratio, showing minimum yield at optimal IS/ MG value

Substituting the value of  $\vartheta/\gamma = 5.6092$  into the model equation (3) gives that the minimum ethanol

yield is 1.8028%. Further increase in  $\vartheta/\chi$  (IS/MG ratio) resulted to increase in the ethanol yield. The model predicts that beyond the IS/ MG ratio; 5.6092, ethanol yield increases as the ratio increased. This suggests that the incremental trend for inoculums size is much higher compared to that of microbial growth. It is suspected that the opposite was inherent before minimum ethanol yield was reached. This comparative analysis of the incremental trends was achieved by substituting assumed values of  $\vartheta/\chi$ ; 6.234, 7.1065, 12.4321, 18.6421 and 25.4566 into the model equation (3) to predict the corresponding ethanol yields. These values were plotted as shown in Fig. 7.

**Deviational Analysis:** Analysis of the ethanol yield obtained from the actual and model-predicted results shows deviation on the part of model-predicted results. This was attributed to the fact that the effects of the surface properties of the substrate which played vital roles during fermentation were not considered during the model formulation. This necessitated the introduction of correction factor, to bring the model-predicted ethanol yield to those of the corresponding experimental values.

The deviation  $Dv$ , of model-predicted ethanol yield from the corresponding actual result was given by;

$$Dv = \left( \frac{\xi_P - \xi_E}{\xi_E} \right) \times 100 \quad (13)$$

where,

$\xi_E$  and  $\xi_P$  are ethanol yield evaluated from actual and model-predicted respectively

Fig.8 shows that maximum deviation of model-predicted ethanol yield from the actual results was less than 7.5%. This translates into over 92.5% model operational confidence. The figure shows that the least and highest deviations of model-predicted results (from actual results) are -0.94 and 7.03 %.

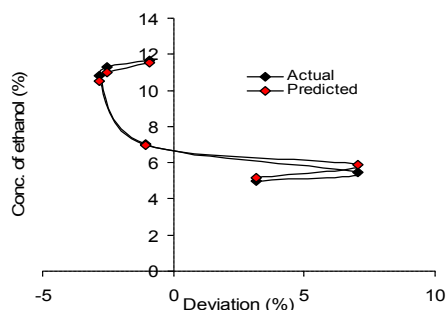


Fig. 8: Deviation of model-predicted results from actual values

These deviations correspond to model-predicted ethanol yield: 11.5207 and 5.8652 (%); inoculums size:

1 and 2.8 (O.D) and microbial growth: 0.98 and 1.782 (O.D) respectively.

Correction factor, Cf to the model-predicted results was given by

$$Cf = - \left( \frac{\xi_P - \xi_E}{\xi_E} \right) \times 100 \quad (14)$$

Critical analysis of Fig. 8 and Fig. 9 show that the evaluated correction factors are negative of the deviation as shown in equations (13) and (14).

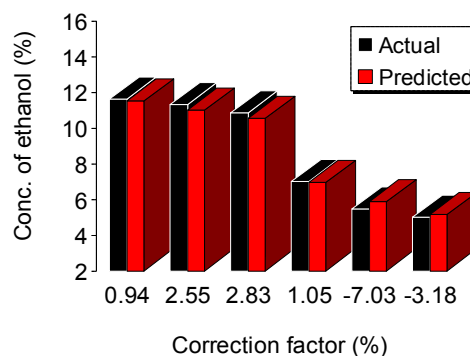


Fig. 9: Correction factor to model-predicted results

The correction factor took care of the negligence of operational contributions of the effects of surface properties of the substrate which actually affected the fermentation process. Introduction of the corresponding values of Cf from equation (16) into the model gives exactly the corresponding actual ethanol yield. Fig. 9 indicates that the maximum correction factor to the model-predicted ethanol yield to the model-predicted ethanol yield was less than 7.5%. The figure shows that the least and highest correction factors to the model-predicted results (from actual results) are 0.94 and - 7.03%. These correction factors also correspond to model-predicted ethanol yield: 11.5207 and 5.8652 (%); inoculums size: 1 and 2.8 (O.D) and microbial growth: 0.98 and 1.782 (O.D) respectively.

The deviation of model predicted results from that of the actual is just the magnitude of the value. The associated sign preceding the value signifies deviation deficit (negative sign) or surplus (positive sign).

## CONCLUSION

The response of machine-driven ethanol yield to the production input ratio of inoculums size and microbial growth has been evaluated. Ethanol yield was almost a quadratic function of the input ratio IS/MG as predicted by the derived empirical model. Ethanol yield

decreases with increased IS/MG ratio in line with experimental result until at a certain value of IS/MG where the ethanol yield drops. Differentiation of the model shows that ethanol production is minimum (1.8028 %) at IS/MG of 5.6092 above which the yield rises. The model;  $\zeta \approx 11.7(\vartheta/\bar{x})^{-1.65} + 0.2(\vartheta/\bar{x}) + 0.001$  predicts the ethanol yield with maximum deviation < 7.5% (from actual results). This translated into over 92.5% operational confidence levels for the derived model. The validity of the model was rooted on the core model expression  $\zeta - S \approx N(\vartheta/\bar{x})^{-K} + H(\vartheta/\bar{x})$  where both sides of the expression are correspondingly almost equal. The standard error incurred in predicting the ethanol yield relative to values of the actual results was 0.16%. The correlation coefficients between ethanol yield and inoculums size, microbial growth and IS/MG ratio were all > 0.88.

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