

Research Article

## Optimization of the production of Bioethanol from waste Newspaper using Yeast isolate from Palm oil polluted soil

Ifeanyichukwu Edeh\* and Obinna Ezeibe

Department of Chemical Engineering,  
University of Port Harcourt, Nigeria

\*Email of the Corresponding author: ifeanyichukwu.edeh@uniport.edu.ng

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### Abstract

The high cost of bioethanol resulting from the uneconomical price of feedstocks has led to the investigation of potential feedstocks from inedible substances such as lignocellulosic materials including waste biomass. One of the problems of producing bioethanol from waste biomass is low yield coupled with the high cost of organic catalysts. The current work was geared towards optimizing the concentration of bioethanol from the fermentation of waste newspapers using yeast isolate from palm oil polluted soil. The optimization was conducted using Central Composite Design in Design Expert software. The experimental parameters considered included pH, inoculum size, incubation time and carbon:nitrogen ratio, and the results obtained show that quadratic models best fitted bioethanol, yeast biomass and reducing sugar experimental data. The interaction of inoculum size and incubation time impacted most on the concentrations of the bioethanol, yeast biomass and reducing sugar. The optimum concentrations of bioethanol (30.12 mg/mL), yeast biomass (0.82 mg/mL) and reducing sugar (3.0 mg/mL) were obtained using operating condition of pH (7), inoculum size (6 %), inocubation time (14 days) and carbon:nitrogen ratio (52). The bioethanol being produced from waste feedstock, and yeast isolated from palm oil polluted soil could be more economical than the conventional gasoline, and the process developed could serve as a sustainable waste newspaper disposal management.

**Keywords:** Bioethanol; yeast, optimization; waste newspaper; palm oil

### 1.0 Introduction

The interest in bioethanol has grown over the past decade due to its potential to substitute for the conventional gasoline, which has several environmental and climate-related concerns [16]. The Kyoto Protocols and most recently, Paris Protocols advocate for the use of clean, green and renewable transportation fuels to replace gasoline, diesel and jet fuel [19]. Bioethanol for use in motor vehicles are considered a potential alternative for carbon emission savings since its production processes ensures significant reduction in net emissions [8].

One of the challenges of bioethanol production have been the issue of choice of feedstock. In this study, waste newspaper was used as feedstock for the production of bioethanol. The choice of waste newspaper is occasioned by its availability and cost effectiveness [9]. Production of bioethanol from waste papers presents an economically competitive alternative source of fuel to petrol. Biomass feedstock cost is the main contributor to bioethanol cost [1]. Moreover, paper disposal is a well-documented environmental challenge [3]. Therefore, converting paper biomass to

energy feedstock plays two vital roles: a) provision of a cheap and available feedstock for bioethanol production and b) reducing the pressure on the depleting landfills. The choice of waste newspaper is an effort to avoid the problem of food and feed crops challenge as feedstock for bioethanol production. There is also the problem of managing paper waste arising from the shrinking of landfill capacity due to landfill costs which is mainly due to the waste paper from the municipal waste [21]. Many studies have focused on the production of bioethanol using paper due to this concern. Wang *et al.* [22] reviewed the production of bioethanol from waste papers, namely newspaper, magazine paper, office paper and cardboard paper with the discovery that bioethanol from all these waste papers can be economically more attractive than petrol at pump price. *Saccharomyces* sp. was used in the production of bioethanol from paper. Literature on the production of bioethanol by *Saccharomyces* sp. abound. *Saccharomyces* sp. is the most reported organism in bioethanol production. *S. cerevisiae* is superior to bacteria, other yeasts, and filamentous fungi in various physiological characteristics regarding ethanol production in industrial context. It tolerates a wide range of pH with acidic pH as optimum; this ensures that its fermentation process is less susceptible to infection than bacteria. It also tolerates ethanol better than other ethanol producing microorganisms *Saccharomyces* sp are classified as GRAS (meaning “generally regarded as safe” for human consumption which enhances its advantageous utilization more than other yeasts and microorganisms [15,11]. Based on the low concentration of the bioethanol obtained by Edeh and Ezeibe, [6], the current study is focused on optimizing the bioethanol production from the fermentation of waste newspaper using yeast isolate from palm oil polluted soil.

The conditions studied were pH, incubation time, inoculum size and carbon-nitrogen ratio.

## 2.0 Materials and Methods

### 2.1 Materials

#### 2.1.1 Sample collection

The oil sample was collected from palm oil mill in Obio/Akpor Local Government Area of Rivers State, Nigeria. The waste newspapers were collected from the archive in a private residence at Obio-Akpor Local Government Area, Rivers State, Nigeria.

#### 2.1.2 Chemicals

The Chloramphenicol and Corn kernel were purchased from CAPPINO Company and local market in Alakahia, respectively. The following chemicals were bought from JOECHEM Chemicals, Rivers State, Nigeria: Sodium sulphate (99% pure, Guangdong Chemical Reagent Engineering-technological Research and Development Center Product), the Mineral Salt media used was Bushnell Haas Broth (T.M MEDIA Product), Potassium Iodide (99% pure), Potassium sulphate (99% pure), sulfuric acid (98% pure), Sterile normal saline (0.9% - 9g of salt per litre), Dinitrosalicylic acid (DNS), Sodium hydroxide (97% pure), Potassium dichromate (99% pure), Iodine (98% pure), Calcium alginate gel, mercuric oxide, Petroleum ether, Hydrochloric acid, Paraffin wax. T, and Glucose Yeast Peptone media used was Potato Dextrose Agar.

#### 2.1.3 Equipment/apparatuses

These include Thermostat Oven (DHG-9023A Model), Vacuum pump and Buchner funnel/flask, pH meter (HANNA Instruments with range 0.0 to 14.0 pH and resolution 0.1 pH), Stainless Steel Portable Pressure Sterilizer (YX-280A Model), G and G Analytical Weighing Machine (JJ300 Model), Thermostat Incubator (DNP-9022-1A Model), and B-Bran Microscope (XSZ-107BN Model),.

## 2.2 Methods

### 2.2.1 Enriching the palm oil contaminated soil samples

The soil sample was enriched in Mineral salt media (Bushnell Haas media-BHM) using palm oil as carbon source. 90mL of the Bushnell Haas media and 10 mL of palm oil were put in 250mL conical flask. The sterilization was monitored at 121°C and 15 psi for 15 min. The palm oil further underwent vacuum filtration, then the setup was aerated and vortexed using an orbital shaker incubator at 170 RPM and 37°C for 30 min [7].

### 2.2.3 Isolation of yeast species from palm oil polluted soil

9.9 mL of sterile normal saline was used to dilute 2g of the enriched soil sample, before adding glucose yeast peptone media which was prepared using 10 g/L of glucose, 2.5 g/L of yeast extract, 7.5 g/L of agar and 0.05 g/L of tetracycline [17,18]. The mixture was put in petri dishes and autoclaved at 121°C and 15 psi for 15min. The vacuum filtered palm oil obtained from the enrichment experiment was used and the vapour-phase culturing technique was adopted by soaking the sterile Whatman filter paper no1 with palm oil and aseptically placed on the cover of the petri dishes. The plates were incubated at 37°C for 48 hr. Yeast flora was determined by inhibiting bacterial contaminants using 1.0µg/mL chloramphenicol [14].

### 2.2.4. Identifying the yeast isolates obtained from palm oil contaminated soil

The method of McFaddin [13] for production of urease as a means of determination of the identity of yeast was used. With a wire loop, a purified colony of the yeast isolate was streaked on a Christensen's urea agar plate. The plate was incubated at 37°C for 24hr. The medium was examined for colour change; a change from amber to pink or red colour signifies positive results while no colour change signifies negative results.

### 2.2.5 Preparation of corn steep liquor for nitrogen enrichment

200g of corn kernel was spread in an open container, and was sprayed with deionized water before covering. This was left for 48hr at room temperature. The corn kernel was ground into powder form with a grinding machine, and soaked in water in the ratio of 1:10 (wt/v). The resulting solution was sieved with a 0.2mm diameter mesh and the liquor obtained and frozen for subsequent use during fermentation.

2.2.6 Determination of the reducing sugar  
Standard dilutions of the reducing sugar were put in seven different tubes, and 3mL of DNSA reagent was added to each of them including a blank at room temperature and plugged with cotton wool. The test tubes were placed in a boiling water bath for 15 min before cooling at room temperature. After which the absorbance of the samples was determined using a spectrophotometer at a wavelength of 540 nm. The concentration of the samples were obtained using a calibration curve developed.

### 2.2.7 Pre-treatment of waste newspaper

This was conducted to disintegrate the fibres of the newspaper and make it easier to separate the cellulose which is the predominant constituent of the newspaper. Waste newspapers were cut manually into small pieces using scissors to increase the surface area. 300g of these shreds were weighed and soaked in 500mL of distilled water for 24hr.

### 2.2.8 Hydrolysis of waste newspaper

Acid solvent hydrolysis was carried out by dispensing 100 g of the pre-treated newspaper material into a 250mL Erlenmeyer flask, and adding 200mL of sulfuric acid solvent. The mixture was placed in an autoclave at 121°C and 15psi for 15minutes [12]. The pH of the mixture was adjusted by reacting with 1.0N sodium hydroxide. After adjusting the pH, the mixture was drained and the resulting sample was filtered using a vacuum

filtration apparatuses to obtain the hydrolysate. The material was dried in an oven and used as substrates for the fermentation.

2.2.9 Fermentation of the hydrolysate leading to the production of bioethanol

The experiment was conducted using the experimental conditions presented in Table 1. 100 g/L of the must was used for the fermentation of the yeast strain. Corn steep liquor was added to the must in varying quantities during each assay for nitrogen enrichment. In addition, 10% fermentation volume was used in water at 35°C for diluting the yeast for 30 min before adding to samples for fermentation. Wine filtration

process with filter pads/membrane was then used to remove yeast and other particles. The bioethanol was recovered from the fermentation broth by distillation at a temperature of 78°C.

2.2.10 Design of Experiment (DOE) and statistical analysis

The DOE was carried out upper and lower bounds of the process variables (pH, inoculum size, inoculation time and carbon:nitrogen ratio). The pH (6 - 9), inoculum size (2 - 10 %), inoculation time (10 - 18 days), and carbon:nitrogen ratio (48 - 56) were used in the design to produce the experimental conditions presented in Table 1.

Run	A:pH	B: Inoculum size (%)	C: Incubation time (days)	D: Carbon:nitrogen ratio
1	9	6	14	52
2	8	4	12	54
3	7	6	14	52
4	6	8	16	54
5	6	4	16	54
6	7	6	10	52
7	7	6	14	52
8	7	6	14	52
9	8	8	16	50
10	7	6	14	52
11	7	6	14	52
12	7	10	14	52
13	8	4	16	50
14	8	8	12	54
15	8	4	12	50
16	6	4	12	50
17	6	4	12	54
18	7	6	14	52
19	7	6	14	56
20	8	8	12	50
21	6	4	16	50
22	8	4	16	54
23	7	6	14	48
24	7	6	18	52
25	5	6	14	52
26	6	8	12	50
27	7	2	14	52
28	6	8	12	54
29	8	8	16	54
30	6	8	16	50

**Table 1.** Experimental conditions obtained using the Central Composite Design for the production of bioethanol from waste newspaper using yeast isolate from palm oil polluted soil

Regression analysis was conducted on the experimental data, and quadratic models of the response (bioethanol and yeast biomass production, and the reducing sugar) conscripted from the one shown in Equation 1 were obtained.

$$Y = \beta_2 + \beta_1A + \beta_2B + \beta_3C + \beta_4D + \beta_{11}A^2 + \beta_{22}B^2 + \beta_{33}C^2 + \beta_{44}D^2 + \beta_{12}AB + \beta_{13}AC + \beta_{14}AD + \beta_{23}BC + \beta_{24}BD + \beta_{34}CD \quad (1)$$

where Y: Predicted response (biomass and bioethanol production);  $\beta_2$ = intercept,  $\beta_1 - \beta_4$  = main effect,  $\beta_{11} - \beta_{44}$  = quadratic effect and  $\beta_{12} - \beta_{34}$  = interactive effect

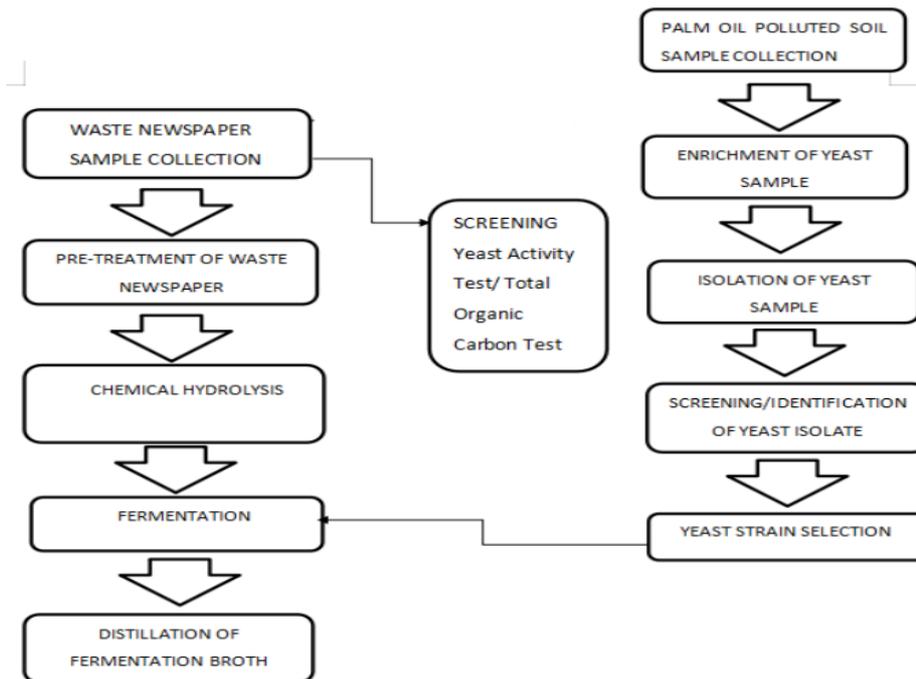
The models obtained were fitted to the experimental data and analyzed using analysis of variance (ANOVA) and residual. The model graphs of the variable interactions effect are presented.

### 2.2.11 Yeast biomass estimation

This was carried out using the dry cell weight measurement method. 10mL of the yeast cell suspension was put into 30mL centrifuge tube, and centrifuged for 15mins at 4000rpm. The resulting mixture was decanted and the supernatant having the yeast cells were washed twice with iced distilled water by centrifugation for another 15mins at 4000rpm. The cell paste was removed from the centrifuge tubes and placed on the weighing pan. Weights of the pan plus the weight of the cell paste were measured and recorded. The cell paste was oven dried for 6-24hr at 105°C and allowed to cool in a desiccator. The weight of the pan plus the dried cell paste was periodically taken, until the weight became constant. The dry weight was quantified based on gravimetry and expressed in mg/L.

### 2.3 Summary of the experimental methods

The summary of the experimental methods for the production of bioethanol from waste newspapers using yeast isolate from palm oil polluted soil is presented in Figure 1.



**Figure 1.** Flow chart for Experimental Procedure for Production of Bioethanol from Waste Newspaper using Yeast Isolate from Palm Oil Polluted soil

## 3.0 Results and Discussion

### 3.1 Isolation of yeast species from palm oil contaminated soil

The efficacy of the Five yeast isolates from the palm oil contaminated soil to degrade waste newspapers over a period of 7 days was assessed. Two of the yeast isolates (Pops 1 and Pops 2) gave a positive result as confirmed by the plate hydrolysis.

The result of the biochemical analysis of the yeast isolates is presented in Table 1.1. As shown in the figure, the yeast demonstrated varying degrees of sugar fermentation with all the isolates fermenting glucose and galatose which is a typical characteristics of the genera

*Saccharomyces sp.* It shows two strains (Pops 2 and Pops 4) fermented sucrose unlike the other five. The isolates also varied in the capacity to utilize urea, with only three (Pops 1, Pops 3 and Pops 5) showing ability to utilize urea.

**Table 1.1.** Biochemical characteristics of yeast isolates from palm oil impacted soil

Isolate code	Lactose	Sucrose	Galactose	Glucose	Urea	Probable Genera
Pops 1	+	-	+	+	+	<i>Saccharomyces sp</i>
Pops 2	-	+	+	+	-	<i>Saccharomyces sp</i>
Pops 3	+	-	+	+	+	<i>Saccharomyces sp</i>
Pops 4	-	+	+	+	-	<i>Saccharomyces sp</i>

### 3.2 Response surface optimization of the bioethanol production

This was carried out by conducting the experiment based the experimental conditions obtained from the CCD using Design Expert. The responses of bioethanol, biomass and reducing sugar obtained from the experiments at the experimental conditions are presented in Table 2. The table shows that optimum operating conditions of pH (7), inoculum size (6 %), incubation time (14 days) and carbon:nitrogen ratio (52)

**Table 2.** Design and Response Plot for bioethanol production

Run	A: pH	B: Inoculum size (%)	C: Incubation time (day)	D: Carbon: Nitrogen	Response		
					Bioethanol (mg/kg)	Yeast biomass (mg/mL)	Reducing sugar (mg/mL)
1	9	6	14	52	7.43	0.11	0.5
2	8	4	12	54	21.56	0.41	2
3	7	6	14	52	28.22	0.72	1.5
4	6	8	16	54	18.21	0.23	2.6
5	6	4	16	54	15.82	0.19	1
6	7	6	10	52	7.94	0.11	1.2
7	7	6	14	52	28.22	0.62	2.7
8	7	6	14	52	26.12	0.72	2.5
9	8	8	16	50	5.89	0.08	1
10	7	6	14	52	27.22	0.52	2.6
11	7	6	14	52	28.12	0.82	2.8
12	7	10	14	52	14.74	0.21	1
13	8	4	16	50	5.36	0.16	1.3
14	8	8	12	54	6.57	0.23	1.7
15	8	4	12	50	3.91	0.09	0.6
16	6	4	12	50	17.32	0.37	2.1
17	6	4	12	54	26.78	0.54	1.8
18	7	6	14	52	30.12	0.82	3
19	7	6	14	56	16.72	0.48	1.9
20	8	8	12	50	4.64	0.18	0.6
21	6	4	16	50	9.25	0.12	1.4
22	8	4	16	54	14.39	0.31	1.6
23	7	6	14	48	10.24	0.26	1.6
24	7	6	18	52	8.78	0.3	1.2
25	5	6	14	52	17.84	0.52	1.4
26	6	8	12	50	11.52	0.27	0.8
27	7	2	14	52	17.46	0.43	0.4
28	6	8	12	54	18.93	0.34	0.32
29	8	8	16	54	9.54	0.13	0.7
30	6	8	16	50	13.61	0.12	1.8

gave the maximum bioethanol (30.12 mg/mL), yeast biomass (0.82 mg/mL) and reducing sugar (3.0 mg/mL) from the experiment. Comparing this result with the maximum bioethanol (0.27 mg/mL) and yeast biomass (0.60 mg/mL) concentrations obtained by Edeh and Ezeibe [6] using an inoculum size of 6 % and pH 7 after 8 days of incubation shows an increment by times (×) 111. Since, the source of yeast is the same, the increased performance of the fermentation is attributed to the application of favourable operating conditions such as conducting the experiment for 14 days instead of the 8 days used by Edeh and Ezeibe [6]. The concentration of the ethanol is greater than 3.73 ±0.16 g/L obtained by Dubey et al. [4] using *Pichia Stipitis*, and this may be due to the difference in the source of microorganism and the experimental conditions used.

### 3.1.1 . Model development

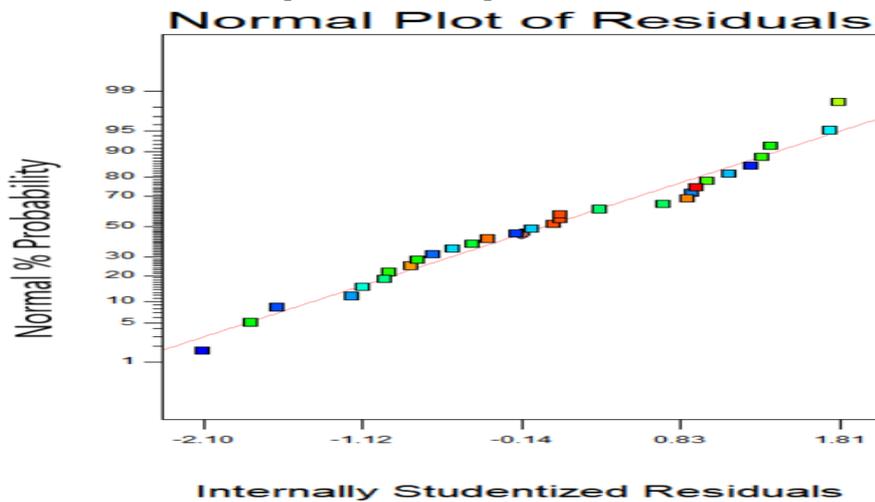
#### (1) Bioethanol response

The quadratic model obtained is presented in Equation 1. The model was validated using the Analysis of variance (ANOVA) and residual. The result shows that the model is significant as the prob>F was less than 0.05. The “Lack of Fit F-value” of 4.85 Implies that Lack of fit is significant. The “Pred R-Squared” of 0.7442 is in

$$\text{Bioethanol} = 25.37 - 10.77A - 3.31B - 0.45C + 6.37D - 2.19AB + 3.03AC + 0.79BC - 6.28BD - 3.15CD - 8.62A^2 - 11.86B^2 - 19.60C^2 - 14.48D^2$$

(2)

Reasonable agreement with the “Adj R-Squared” of 0.9077. The significant model terms are A, B, D, BC, BD, A<sup>2</sup>, B<sup>2</sup>, C<sup>2</sup>, and D<sup>2</sup>. Based on the statistical analysis, the model was valid and a good fit for the experimental data. The normal plot of residuals shown in Figure 2, also suggests that the model is a adequate to fit the experimental



**Figure 2.** Bioethanol response Normal Plot of Residuals data as all the points aligned close to the straight line on the plot.

#### (2). Yeast biomass response

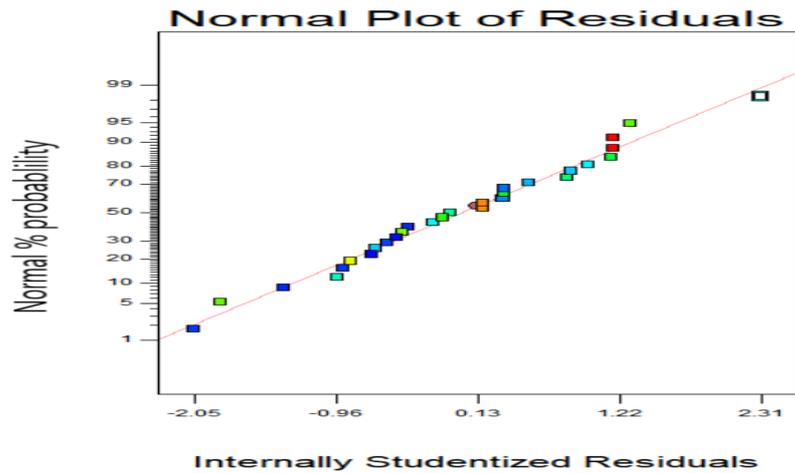
The quadratic model for the yeast biomass produced during the fermentation of waste newspaper by *Saccharomyces* sp is presented in Equation 3.

$$\text{Yeast biomass} = 0.65 - 0.25A - 0.093B - 0.02C + 0.13D - 0.017AB + 0.12AC + 0.028AD + 0.043EC - 0.11BD - 0.058CD - 0.24A^2 - 0.43B^2 - 0.54C^2 - 0.38D^2$$

(3)

Upon statistical analysis, the F-value of 6.21 shows that the model is significant. The significant model terms are A, D, A<sup>2</sup>, B<sup>2</sup>, C<sup>2</sup> and D<sup>2</sup>. These indicate that model is valid and can be used to describe the experimental data. The normal plot of residuals shown in Figure 3,

also suggests that the model is a adequate to fit the experimental data as all the points aligned close to the straight line on the plot.



**Figure 3.** Yeast biomass response Normal Plot of Residuals

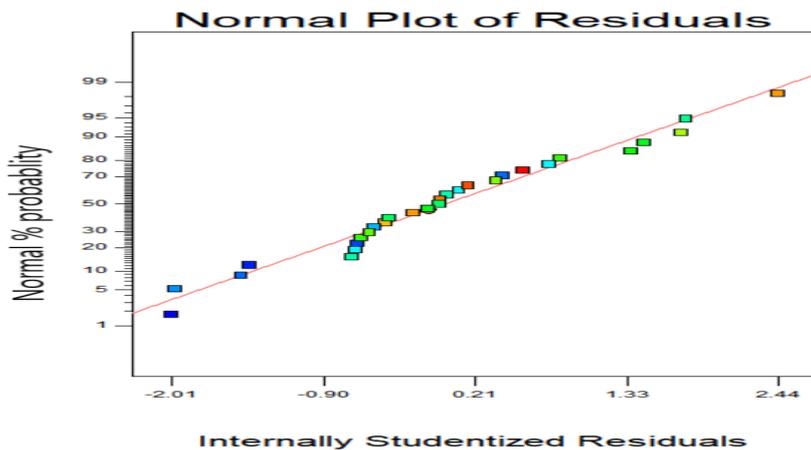
(3). Reducing sugar response

The quadratic models obtained from CCD for reducing sugar release during batch fermentation of paper by *Saccharomyces* sp. is presented in Equation 4

$$\text{Yeast biomass} = 2.53 - 0.88A - 0.14B - 6.667E - 003C + 0.41D - 0.11AB - 0.39AC + 0.54AD + 0.97BC + 0.030BD - 0.33CD - 0.93A^2 - 1.90B^2 - 1.40C^2 - 0.85D^2$$

(4)

The model was validated using ANOVA and it was shown to be significant. The F-value was 3.06 meaning that the model was significant. The significant main effects were A and the squares of A, B, and C. The normal plot of residuals shown in Figure 4, also suggests that the model is a adequate to fit the experimental data as all the points aligned close to the straight line on the plot.



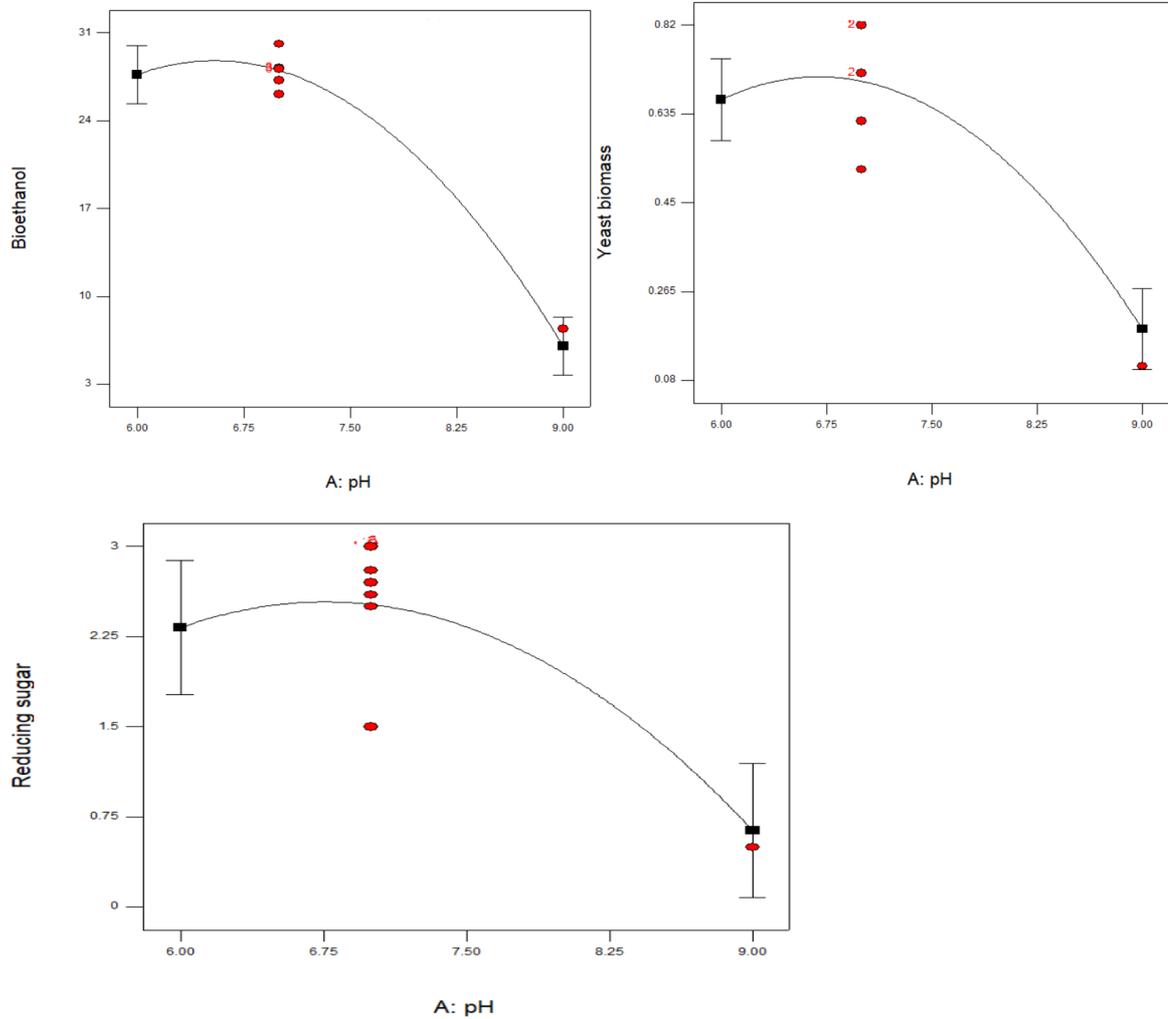
**Figure 4.** Reducing sugar response Normal Plot of Residuals

3.1.2 Effect of single factors on the bioethanol, yeast biomass and reducing sugar obtained during bioethanol production from the fermentation of waste paper using the yeast isolate from palm oil contaminated soil

1. Effect of pH

As shown in Figure 5, pH had an influence over the bioethanol, yeast biomass and reducing sugar obtained from the fermentation of waste newspaper using yeast isolate from palm oil polluted soil at constant inoculum size (6. %), incubation time (14 days) and carbon:nitrogen ratio of 52. The figure shows that the concentrations of bioethanol, yeast biomass and

reducing sugar decrease with pH with the highest of 30.12 mg/mL, 0.82 mg/ mL and 3 mg/mL), respectively, obtained using a pH 7 at constant operating conditions of inoculum size (6 %), incubation time (14 days) and carbon:nitrogen ratio of 52. The minimum concentrations of bioethanol, yeast biomass and reducing sugar were 7.43 mg/mL, 0.11 mg/mL and 0.5 mg/mL obtained



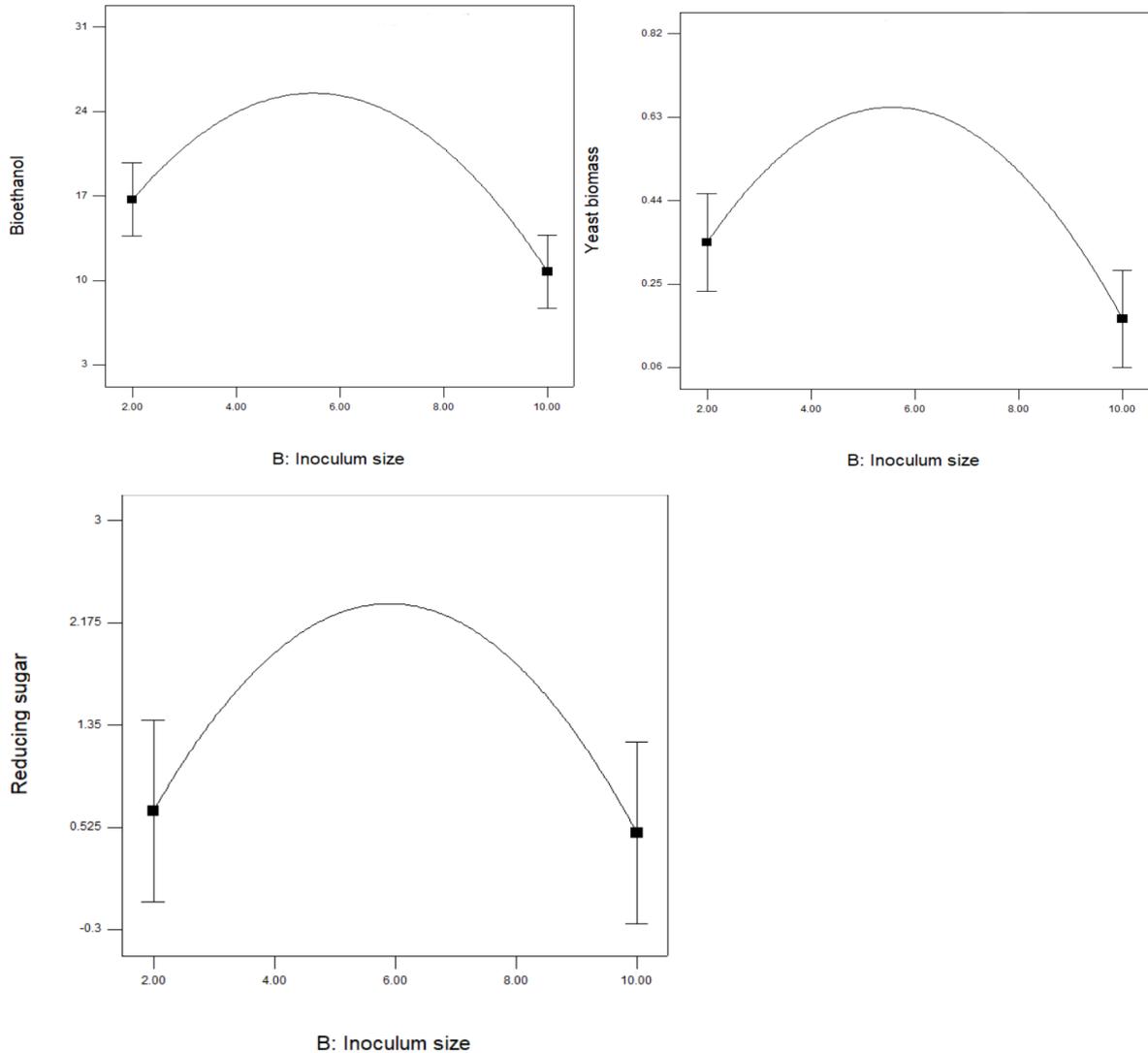
**Figure 5.** Effect of pH the bioethanol production from the fermentation of waste paper using the yeast isolate from palm oil contaminated soil

Using pH 9 at operating condition of inoculum size (6 %), incubation time (14 days) and carbon:nitrogen ratio of 52. The decrease in concentration of bioethanol, yeast biomass and reducing sugar with pH shows that the yeast cells cannot perform optimally in an environment with pH greater than 7 as they might not survive due to chemical stress [2,5]. The pH 7 giving the maximum concentration of the fermentation products could mean that this pH provided a balance between cellular growth and the physicochemical impact.

## 2. Effect of inoculum size

The investigation of the effect of the inoculum size (2 - 10 %) on the bioethanol, yeast biomass and reducing sugar production during the fermentation of waste newspaper using yeast isolate from palm oil polluted soil revealed an optimum inoculum size of 6 % at

constant pH (7.50), incubation time (14 days) and carbon:nitrogen ratio (52). This condition resulted to about 24 mg/mL, 0.63 mg/mL and 2.175 of bioethanol, yeast biomass and reducing sugar, respectively (see Figure 6). Edeh and Ezeibe [6] also, obtained an increment in the concentration of bioethanol (0.27 mg/mL) and yeast biomass (0.60 mg/mL), but, after 8 days of incubation. The difference in the result could be attributed to the variation in the operating conditions.

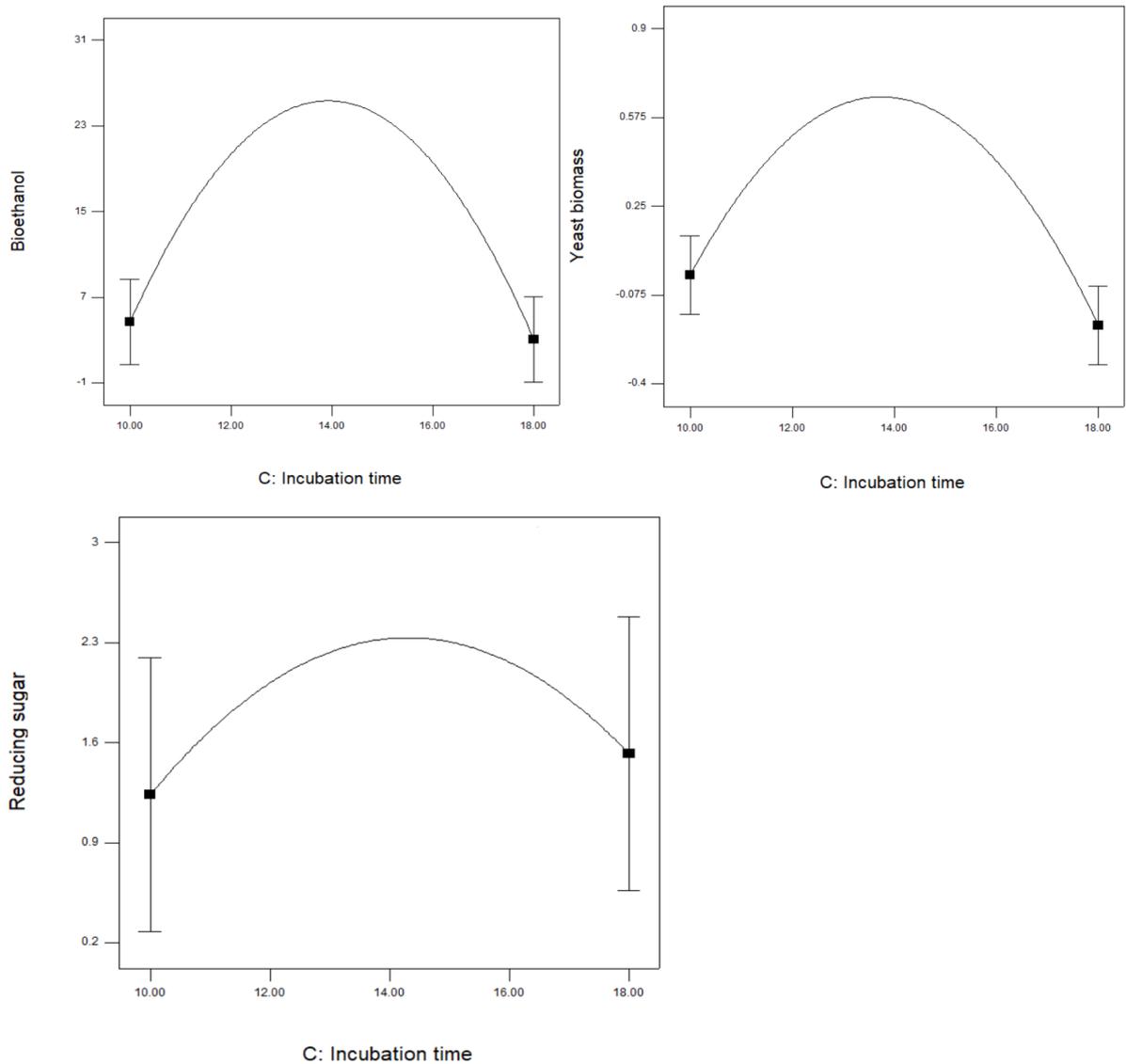


**Figure 6.** Effect of inoculum size the bioethanol production from the fermentation of waste paper using the yeast isolate from palm oil contaminated soil

As shown in Figure 6, there was a decline in the fermentation products using inoculum size greater than 6%. This may be attributed to the suspected depletion of nutrients [10].

### 3. Effect of incubation time

The result obtained from the evaluation of the effect of the incubation time on the fermentation of waste newspaper using yeast isolate from palm oil polluted soil on bioethanol, yeast biomass and reducing sugar products is presented in Figure 7. The figure shows that the concentration of the products increased with inocubation time until after 14 days when it started to decline.

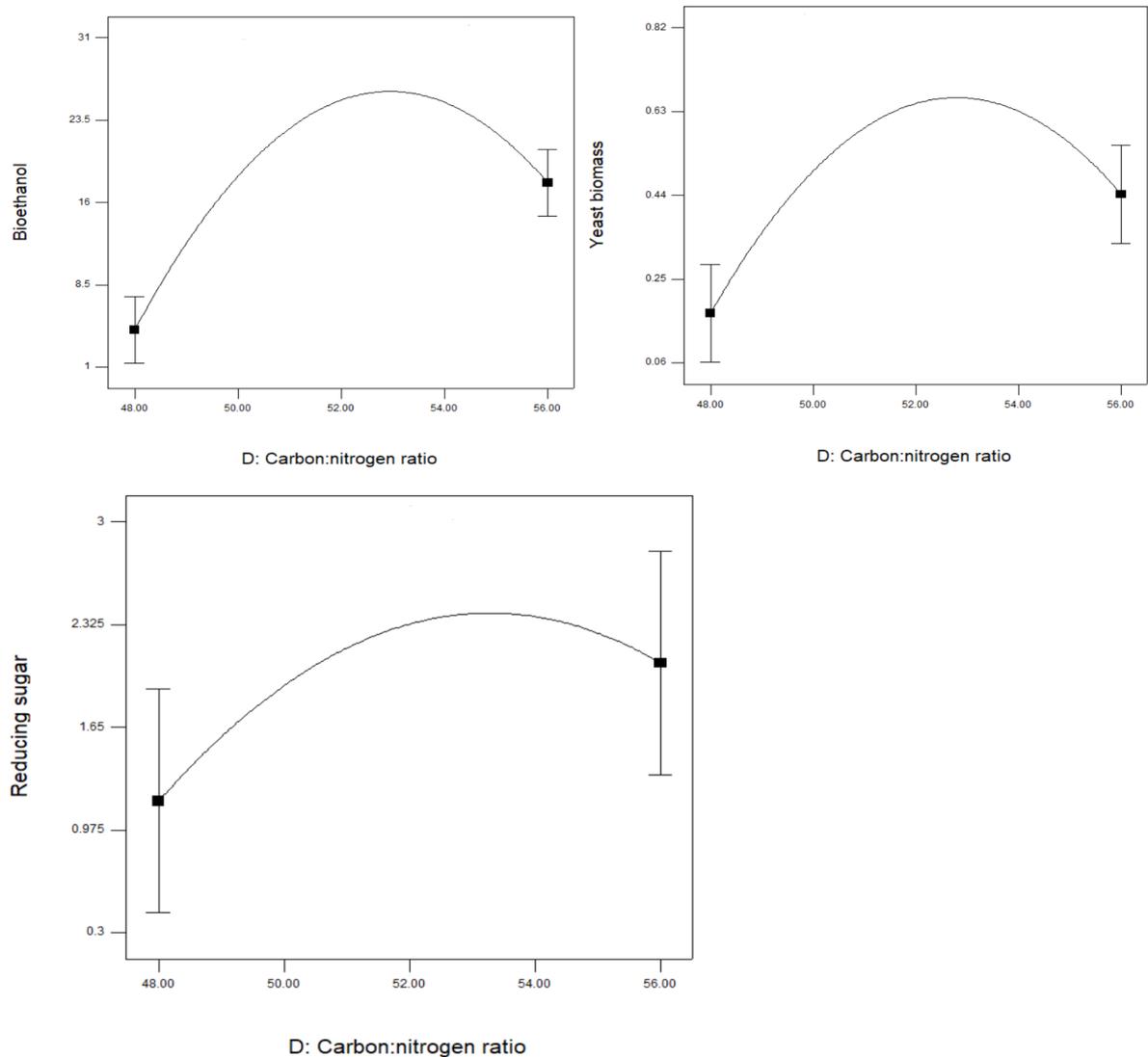


**Figure 7.** Effect of incubation time the bioethanol production from the fermentation of waste paper using the yeast isolate from palm oil contaminated soil

This result could be due to the increase in the growth of the yeast biomass and the quantity of the reducing sugar produced as the incubation time increases, although, after 14 days, a decline was observed as the high concentration of bioethanol is suspected to become toxic to the yeast cells Viegas and Sa-Correia [20].

#### 4. Effect of carbon:nitrogen ratio

Figure 8 shows that increase in the carbon:nitrogen ratio (48 and about 53) increases the production of bioethanol, yeast biomass and reducing sugar.

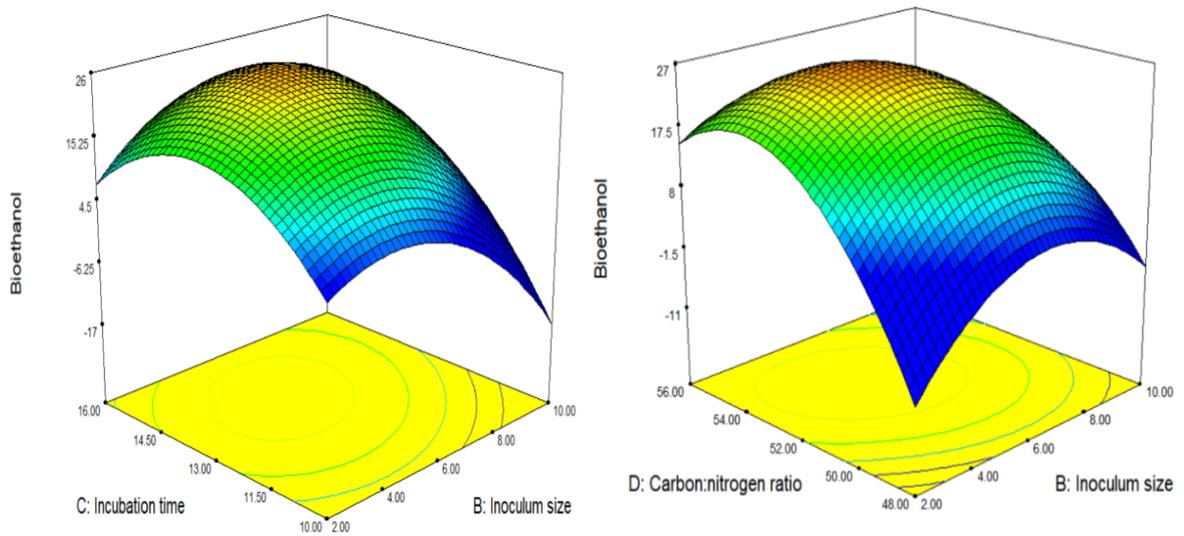


**Figure 8.** Effect of carbon:nitrogen ratio on the bioethanol production from the fermentation of waste paper using the yeast isolate from palm oil contaminated soil

Beyond a carbon:nitrogen ratio of approximately 53, the production of bioethanol, yeast biomass and reducing sugar decreases under the operating condition of pH (7.5), inoculum (6 %) and incubation (14 days). The increase in the carbon:nitrogen ratio means that the amount of carbon source (waste newspaper) was higher than the nitrogen source (corn steep liquor) leading to the increase in concentration of bioethanol, until when a ratio beyond 53 was attained causing a decline in the production of the bioethanol. This decline may be attributed to the toxification of the yeast cells Viegas and Sa-Correia [20].

### 3.1.3 Effect of factor interaction on the products obtained from the fermentation of waste paper using the yeast isolate from palm oil contaminated soil

The effect of the interaction of the operating parameters on the production of bioethanol, yeast biomass and reducing sugar was assessed. The parameters evaluated were pH, inoculum size, incubation time and carbon:nitrogen ratio. Based on statistical analysis, the interactions of the carbon:nitrogen ratio and inoculum size, and carbon:nitrogen ratio and incubation time had a significant effect on the bioethanol production as shown in Figure 9. The interaction of incubation time and inoculum



**Figure 9.** Effect factor interaction on the bioethanol production from the fermentation of waste paper using the yeast isolate from palm oil contaminated soil

Size gave the highest concentration of bioethanol (30.12 mg/mL) under the operating condition of pH (7.50), and carbon:nitrogen ratio (52). This performance is greater than the result obtained when the incubation time and inoculum size were investigated individual, as shown previously.

#### 4.0 Conclusion

The optimization of the production of bioethanol from waste newspaper using yeast isolate from palm oil contaminated soil has been investigated. The quadratic models developed for the bioethanol, yeast biomass and reducing sugar production were significant. The interaction of inoculum size and incubation time had the greatest on the performance of the fermentation products. Based on the result obtained, the optimum operation condition of pH (7), inoculum size (6 %), incubation time (14 days) and carbon:nitrogen ratio (52) gave the maximum concentrations of bioethanol (30.12 mg/mL), yeast biomass (0.82 mg/mL) and reducing sugar (3.0 mg/mL).

The result shows that waste newspapers is a potential economic and available feedstock for bioethanol production, especially as the first generation bioethanol is expensive due to high cost of feedstock. This will also help to ensure a sustainable waste newspaper disposal management and reduce the emission of greenhouse gas (methane) from the landfill. The use of yeast isolated from a palm oil polluted soil could help to reduce the overall cost of bioethanol production in view of the expensive organic catalysts

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**Conflict of Interest:** No potential conflict of interest declared.

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