# UNDERLYING FACTORS TO CONSIDER IN IMPROVING ENERGY YIELD FROM BIOMASS SOURCE THROUGH YEAST USE ON HIGH-PRESSURE HOMOGENIZER (HPH)

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## Abstract

Pioneering the works of Brookman (1975), Middelberg et al. (1992a, 1992b) and Kleinig and Middelberg (1996), on cell disruption of yeast through high pressure homogenizer (HPH), the underlying factors in improving energy yield from biomass source has to be considered. This has become a global issue for scientists, researchers and policy makers as the energy demand has grown over the years due to the growing population. As cleaner energy has become highly needed for save environment and protection of the climate hence shifting away from the utilization of fossil fuels will be of higher priority.

In this paper, these factors will be highlighted and discussed herein as well as other parameters that influence the energy production efficiency from the high-pressure homogenizer (HPH) through using yeast as a biomass source. The HPH for consideration in this study is the GYB40-10S; this has a pressure of up to 100 MPa with two stage homogenizing valves pressure. This is adjustable so as to produce superfine, homogenous, stable liquid-liquid or solid-liquid under multiple actions of cavitation effect and high speed impact. And also shear through the adjustable homogenizing pressure valve in the conditions of high pressure and thereby making the material compatible after homogenization.

***Keywords:*** HPH, Biomass, Homogenizing valves, Yeast, Cell disruption, Underlying factors

## 1 Introduction

Energy as the prime mover of economic growth, its search therefore as renewable energy sources in the 21st century has become the key challenge to stimulate a more sustainable energy development for the future [1]. It is one of the most important fundamental elements for human development and even survival [2]. Yeast as one of biomass substrates has increasingly played a dominant role in the production of energy. The continuous production and development of this biomass for energy have been improved on ever since but have also been hampered by the some factors during the production processes. This has made the full potential never to have been achieved till moment. Many studies undertaken from different countries have been recently considering expanding their biofuel production using indigenous resources in order to achieve lower greenhouse gas (GHG) emissions [3, 4].Yeast as an energy producing substrate have been able to meet this target and in the recent advances [5] have been able to show that some microbial species such as; yeast, fungi and microalgae can be used as potential sources for biodiesel as they can biosynthesise and store large amounts of fatty acids in their biomass. Nigam and Singh [6] demonstrated that yeast's ability to grow well on pretreated lignocellulosic biomass could efficiently enhance the lipid accumulation hence providing a promising option for the production of economically and environmentally sound microbial oil from agricultural residues. From previous research, [7] have reported that besides microalgae, many yeasts and fungi species are able to generate and accumulate lipids in their cells. In achieving this breakthrough of liberating the contents within the cell wall of yeast biomass; high-pressure homogenizer was applied in the cell wall disruption at high enough pressure and temperature over number of passes as repeated cycles. In yeast like in other biomasses; Tedesco et al. [8] in their studies analysed the different types of pretreatments performed on various substrates and then considered milling as the most used mechanical technique. This accordingly they discussed in their paper that cell walls and lignin component disruption treatments are needed to enhance the hydrolytic phase and the overall biodegradability of lignocellulosics during an anaerobic digestion process. Similarly, [9, 10] showed pretreatment as a requirement in the alteration of the biomass macroscopic and microscopic size and structure as well as its submicroscopic chemical composition and structure so as to have the hydrolysis of carbohydrate fraction to monomeric sugars to be more achieved rapidly and with greater yields.

Renewable energy use of biomass source constitute the homogenization of yeast under high pressure homogenizer (HPH) and should not be undermined as an energy producing substrate, this in fact, is of great consideration in this study, due to the high content of protein retained within the product and therefore qualifies it as a major and suitable biomass for biogas production. Yeast single cell protein showed molasses which is derived from sugarcane processing as the major raw material for its production [11]. As a result of this; Reed and Nagodawithana [12] have explained that the yeast generated from the biorefinery process has a protein content as high as 50% making it much superior to other co-products from 1G bioethanol production that have been conventionally employed in cattle feed. This in other words, was classed as an energy and protein substitute for grass and with a high protein content containing 31% carbohydrate and lipid as a biomass [12]. Cheirsilp et al. [13] pushed further the frontier of yeast importance by characteristically highlighting it to produce high amount of lipid contents similar to that of vegetable oil as well as having a high growth rate and thus can be cultured in a single medium with low cost substrate. Yeast suitability for biogas production has been stemmed from the fact that it has lower water and higher protein content. Hammerschmidt et al. [14] have explained that several types of waste biomass and fresh plants available for the production of energy and fuels as not suitable for common pyrolysis processes due to their high water content (>70%). For the process to be classical the biomass has to be dried; and this has been considered as energy and time consuming step [14]. Apart from the fact that yeast uses in human's lives are numerous; such as in the industry for brewery, pharmaceutical, food and now, energy production. Yeast as oleaginous microorganisms has an advantage over bacteria, molds and algae due to its unicellular nature as it is having a relatively high growth rate and accumulates lipid rapidly in discrete

lipid bodies [15]. Saenge et al. [16] found and concluded that the produced lipid can be used as a feedstock for biodiesel production and when compared with other vegetable oils and animal fats Li and Wang [17] showed yeast lipid production having many advantages, such as; short life cycle, requiring little labour to grow, easy to scale up as well as relatively independent of special requirements for place, season and climate.

In fact, biomass-based energy sources are potentially carbon dioxide neutral and recycle the same carbon atoms hence bioenergy is termed renewable energy made from plant-derived organic matter which are collectively recognized as biomass [18]. It is therefore important to recognize this fact that developing this biomass energy will largely be dependable on the development of the renewable energy industry as a whole, as it is driven by similar energy, environmental, political, social and technological considerations [19]. The aim of this study was to determine the underlying factors in improving energy yield through using yeast in high-pressure homogenizer and through the conducted study, some significance of these parameters are presented which determines the energy yield after homogenization.

## 2 Background

The disruption of yeast through high pressure homogenizer (HPH) has all been previously dealt with by [20-23] using the different parameters in their analysis. [20] studied the antimicrobial effects and suggested it as dependent on the rate and magnitude of pressure drop. [24-26] proposed high pressure homogenizer as an effective alternative to the pasteurization of milk and whole liquid eggs. Donsi et al. [27] highlighted pressure and temperature as mainly influencing the effectiveness of homogenization for microbial inactivation among the process parameter and contrarily, considered temperature effects to be necessarily taken into account in HPH since upon homogenization, the rise in temperature is observed in the fluid downstream of the valve. Floury et al. [28] therefore attributes this to the viscous stress that have been caused by the high velocity of the fluid flow which is then impinging on the ceramic valve of the homogenizer, leading to the dissipation of a significant fraction of the mechanical energy as heat in the fluid. Considering pressure, temperature and number of passes (cycle) as the main parameters that affect fluid flow in HPH, Diels and Michiels [29] reiterated the level of microbial inactivation caused by the application of high-pressure homogenization increases with the pressure level which is similar with the high hydrostatic pressure (HHP) processes. Other underlying factors of considerable importance which have also been analyzed; authors have suggested a correlation between cell wall structure and high-pressure resistance between the microorganisms and HPH. This indicates that high-pressure homogenization kills vegetative bacteria mainly through mechanical destruction of the cell integrity, caused by the spatial pressure and velocity gradients, turbulence, impingement [30,31] and cavitation [32,33]

Disruption of yeast or other microbial organisms is a key step towards the isolation and purification of many biotechnological products that are present in the interior of cells of the cell walls of micro-organisms [34] and it was reported in the concept presented by Clarke et al. [35] as example of breaking the walls of yeast cells. These were found to be between 5-10 microns in sizes and not limited to just yeast but also applicable to other unicellular micro-organisms of different sizes. During homogenization, cell disruption is accounted for as a result of the non-specific tearing apart of the cell wall which is determined by the physical interaction with the valve slit of the homogenizer, in a balance between the destructive fluid-dynamic stresses and the cells' physical strength [27]. This therefore results in the complete deformation of the cell wall and the protein contents are then liberated.

And as energy demand has grown over the years due to the growing population, there are the needs for this energy production to be improved on, so as to further save the environment and protect the climate from effects of using fossil fuels and nuclear energy. This energy produced under this situation is the biogas (mix of $CO_2$ and inflammable gas; $CH_4$) produced by bacteria conversion of organic matter under anaerobic conditions [15]. And as the present energy system is totally considered as being based on fossil fuels, coal oil and natural gas utilization, it therefore makes up over 85% of the primary world energy production and this is possible through their relative easy accessibility which requires relatively little effort to extract, process, and be delivered to the users [16]. Shifting towards low-carbon world as analysed by [17] therefore requires comprehensive efforts being taken worldwide through mitigating measures within the industrialized countries since large majority of anthropogenic greenhouse gases (GHG) are emitted from their current energy use. This however as also shown that developing countries' shares are on the rise and projected to continue in this rise in the nearest future due to developmental improvement.

On the contrary, [18] explained it as being nonsensical to use the fresh water, fertilisers and arable land resources for the growth of fuel when considers the possibility of food shortages as the populations expands especially when taken into accounts the conversion efficiency and lower energy content, and in complimenting the negative effects of food insufficiency for the growing population hence other organic wastes were sourced for to serve the same purpose for energy generation. Kulshreshtha et al. [19] have studied sustainable use of agricultural resources as a biobased economy and therefore considers it not new since agriculture has the potential of being central to this economy through the provision of source materials as commodity items such as liquid fuels and value added products (chemicals and materials). The use of agricultural resources for renewable and sustainable energy generation are not much of interest but [20] considers the land area required for cultivation of these products, should be a primary criteria for evaluating renewable fuels. In the literature many authors have highlighted the use of arable lands in the production of agricultural products for renewable and sustainable energy production. This in 2006 Kampman et al. [21] explained biofuel production uses about 13.8 Mha agricultural land mainly in the USA, EU, Brazil and China and it is expected that global biofuel and related land use will significantly increase to between 73 and 276 Mha by 2020 depending on some factors such as; global demand of biofuel, feedstock mix, the share of second generation biofuels that are produced from agricultural and wood residues and the agricultural yield of these crops up to the year 2020.

This study considers the use of yeast as the biomass resource for the energy conversion process. Yeast use has become necessitated due to its easily availability. Like other microbes species such as microalgae and fungi, recent advances have shown that they can be used potentially in the production of biodiesel as they can biosynthesise and store large amounts of fatty acids in their biomass Xiong et al. [22]. Though in producing biodiesel from renewable energy source, the quality of oil used has a greater impact on the quality of produced biodiesel. Also in genetic engineering, key enzymes in specific fatty acid produce pathways within lipid biosynthesis and are considered as a promising target for the improvement of both quantity and quality of lipids [22, 23].

Yeast dominant role as a biomass substrate in renewable energy production cannot be over exaggerated. This [24] claimed that yeast's ability to grow well on pretreated lignocellulosic biomass could efficiently enhance the lipid accumulation, and therefore provide a promising option for the production of economically and environmentally quality oil from agricultural residues. As factors to consider, biogas yield from yeast homogenization under HPH has been regarded highly favourable under high pressure and above room temperature. This is because thermochemical biomass conversion involves processes which require much more extreme temperatures and pressures than those found in biochemical conversion systems [24]. Though as ascertained, Farias et al. [25] pushed further that certain essential characteristics differentiate thermochemical process from biochemical process, this includes the flexibility in feedstock that can be accommodated with thermochemical processing and fuel diversity that result at the end.

## 3 High-Pressure Homogenizer (HPH)

Cell rupture is required in the recovering of biological products that are located inside cells. This requires to be done either mechanically or non-mechanically [26]. There are other methods as well that are non-mechanical, which could either be the physical way or the enzymatic way. In general, mechanical methods are non-specific, but their efficiency is higher and application broader in comparison to any of the other methods [27]. This is highly dependent on the nature of the product of interest, the cell or tissue itself, like the extent of the cell's fragility [28]. Cell disruption is considered as the isolation and preparation of intercellular products which is important for use in research and in the industries for manufacturing end products for consumers. In the industries, HPH application is linked to the production of stable emulsions, hence it is widely used in such areas [14, 27, 29-30] apart from it being able to emulsify and disrupt particles into disperse phase of suspension, it has also extensively proven to be suitable for the inactivation of the microbial flora occurring in fruit juices and milk-based beverages [31] especially contributing to the preservation of the freshness and texture attributes, coupled with antioxidant capacity and polyphenols, vitamins and flavonoids content of the product [32]. Viscosity is another parameter also considered as a yardstick for the effectiveness of microbial organisms and this is dependent on the associated viscous stress of the material as well as the shear stresses and the concentration of liquid. Temperature on its own can as well be attributed to the viscous stress caused by the high velocity of the fluid flow, which is then impinging on the ceramic valve of the homogenizer, leading to the dissipation of a significant fraction of the mechanical energy as heat in the fluid, this is due to the fact that as the temperature rises, it is observed in the fluid flow downstream of the valve [33]. Homogenizing yeast at such a high pressure enable the yeast to be fully homogenized wherein the cell walls will be totally broken to liberate the protein. Like in the dairy industry, wherein the sizes of milk globules are reduced, this enable it to have a better appearance and longer shelf life, hence [14] regarded HPH as a machine used for emulsions and suspensions to mix, disperse, and reduce the sizes of the droplets or particles of the disperse phase. Since the original function of the homogenizer was breaking up milk fat globules, there is sometimes a connection between the suggestion put forward for breaking up globules in milk, and the rupture of the walls of cells of yeast [14].

**Fig 1.** GYB40-10S 2-Stage Homogenizing Valves HPH

## 4 Materials and Methods

4.1 GYB40-10S 2-Stage Homogenizing Valves HPH

The machine is made of reciprocating plunger pump and homogenizing valve, with its homogenizing portion made up double stage homogenizing system which includes 1st stage homogenizing valve and 2nd stage homogenizing valve. The two stage homogenizing valve's pressure are adjusted under the scope of nominal pressure and at the same time can also be used separately due to the high-low of homogenizing pressure which directly relate to the speed of materials through the homogenizing valve. The machine is of maximum 100 MPa with a flow rate of 40 L/h and material temperature up to 120 °C.
The experiments were conducted using the conventional GYB40-10S 2-Stage Homogenizing Valves High Pressure Homogeniser (HPH)

4.2 Baker's yeast as a biomass substrate

The baker's yeast block was obtained from Dublin Food Sales in the Glasnevin area of Dublin for High Pressure Homogenization experiment. This was refrigerated between 0 – 4 °C for freshness on the day it was originally collected to keep clean and fresh and to also enable it avoid contamination from other source. It was subsequently broken down into large beaker when ready to be used from the block form weighing 950g. 725ml of solution C was added and then ran under the stirrer until it was completely mixed. Using the following composition; the solutions A, B and C as below where prepared.

4.3 Buffer solutions

**Solution A** (0.1M $KH_2PO_4$ + 0.15M NaCl), this is equivalent of 1litre
13.6g of $KH_2PO_4$ weighed into beaker and dissolved using the deionised water, also; 8.8g of NaCl weighed into beaker and dissolved using the deionised water, both mixed together and filled to the 1 litre mark. This was repeated thrice for 3 litres of solution to be obtained.

**Solution B** (0.1M $K_2HPO4$ + 0.15M NaCl),
4.6g of $K_2HPO_4$ weighed into beaker and dissolved using the deionised water, also; 1.8g of NaCl weighed into beaker and dissolved using the deionised water, both mixed together and filled to the 200 ml mark.

**Solution C** obtained through gradually adding Solution B to Solution A until the pH scale of 5.3 was attained.725 ml of solution C added to the 950g broken yeast and mixed using the electric mixer.

**Fig 2a.** Block of yeast **Fig 2b.** Homogenized yeast

4.4 Centrifugation

The centrifuge closet (Fig 3a.) consists of superspeed refrigerated chamber that hold the GSA (Sorvall GSA Rotor). This is an aluminium superspeed angle rotor with specification as 28 °C tube angle, 13,000 rpm as maximum speed [34]. This is having a relative centrifugal force at maximum speed of 27,500 and a k factor at maximum speed as 2026. With the weight as 14.9kg and size diameter as 31cm, it is having a relatively weight of 580g maximum as the compartment mass weight. The Sorvall Superspeed Angle Rotors; GSA (fig 3b.) is designed for use in the Sorvall RC-2, RC2-B, RC-5 and RC-5B Superspeed Refrigerated Centrifuges [34]. The Sorvall Superspeed Angle Rotor accepts a variety of plastic, glass and stainless steel tubes and bottles. GSA have places for 6 and for the purpose of this study, plastic tubes is used for easy removal after centrifugation to prevent mixing of the settled suspension. Before centrifugation, 100 ml of the homogenized sample was poured into a 1 litre graduated beaker. This was ensured that the 100 ml has the same composition as the rest of the homogenized sample (500 ml). This was diluted through adding 900 ml of the buffer (Solution C) at pH 5.3. This form a second diluted sample for protein concentration and as centrifuge is set to 13,000 rpm and timer for 60 minutes. The temperature is set between 5 and 7 °C. When the operation commence, the cooling is then adjusted gradually to between 5 and 7 °C, this is necessitated because it will not start at room temperature.
After centrifugation, the settled suspension is separated by the liquid drained out and the solid residue in the centrifuge tube discarded. This remain consist of the unbroken yeast cells and cell debris.

**Fig 3a.** Centrifuge machine

**Fig 3b**. GSA Rotor **Fig 3c.** Plastic Tube

4.5 Protein curve preparation and spectrophotometer

The standard protein curve preparation is therefore needed as a determination of the protein concentration. One absolute way in determining the concentration of a protein of any given protein contained in a solution with an unknown concentration of protein is to compare the unknown solution with a set of protein solutions of known concentration. This works by having the curve calibrated and in the process enable to determine the exact protein concentration measure in a solution under investigation. Therefore the absorbance associated with a set of protein solutions of known concentrations is otherwise known as the protein standard curve.

Before setting up the assay, the protein to be used as a standard is decided upon and the range which it is likely to be sensitive is determined. As sensitivity vary from one reagent batch to another, immunoglobulin G (IgG) is frequently used as a standard in Bradford assay the same way as bovine serum albumin (BSA) [35]. With the BSA solutions, 0.1M phosphate buffer, pH 7.0 of each protein to 0.5, 1, 2, 4 and 5 mg/ml were prepared to dilutions. Starting at 100mg/ml and prepared serial dilutions were made using Total Protein Reagent and with the UV procedure a Standard Curve for BSA was prepared omitting 0.25 mg/ml dilution. The absorbance of the resulting solution is measured using the spectrophotometer (Fig. 3d) and the measured absorbance of the protein versus the known concentration of BSA is plotted. The resulting graph will then be the protein standard curve for determining the unknown protein concentration.

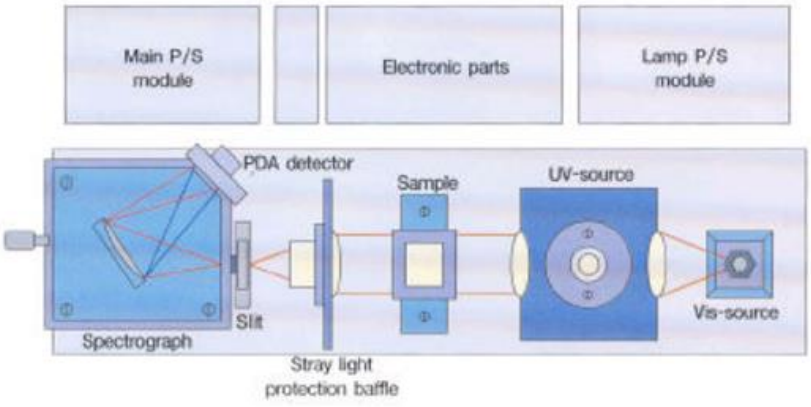


**Fig 3d.** Schematic view of Spectrophotometer S-3100 PDA (As modified from [36])

4.6 Experimental methodologies

The experiment conducted aims at investigating the parameters such as Pressure (P), Temperature (T) and Number of Cycle (N) on the homogenized yeast under High Pressure Homogenizer while at the same considering the other parameter effects on the treated yeast sample such as the pH, Viscosity and Protein Concentration. The HPH; GYB40-10S 2-Stage Homogenizing Valves (as in fig. 1) is a two stage homogenization process The two stage homogenizing valve's pressure are adjusted under the scope of nominal pressure and at the same time can also be used separately due to the high-low of homogenizing pressure which directly relate to the speed of materials through the homogenizing valve. The machine is of maximum 100 MPa with a flow rate of 40 l/h and material temperature up to 120 °C. The functionalities of the valve head, valve seat and impact ring were taken into account during the homogenization as the hand wheel were being turned to compress the yeast through the outlet for yeast cell disruption. The operating guiding principle of pressure effect has previously been explained by [9]. This clarifies the mechanism of cell rupture in terms of the rapid release of pressure as cells pass through the high-pressure homogenizer. The collected homogenized yeast at the different pressures at (30, 60 and 90 MPa), against temperatures (15, 20 and 25 °C) and number of cycles recorded (1, 3 and 5) corresponding to the design matrix as shown below.
The obtained treated yeast and solution is separated from the debris and further diluted with total protein reagent in the ratio of 500ml of the solution (Protein solution) to 2000ml of the reagent for each of the samples. 500ml of this solution is then drawn into each of the cuvettes and allowed to stay for half an hour before the protein value is read using the spectrophotometer. The spectrophotometer is standardized for use at 550 nanometer (nm) wavelengths and the protein concentration is recorded. As spectrophotometer is commonly used for the measurement of transmittance or reflectance of solutions, transparent or opaque solids, such as polished glass, or gases, hence the UV light transmitted through the solution is determined through recording the of the protein [37]

4.7 Design of Experiments

This paper studies the different input parameters that are considered as underlying factors in improving energy yield from biomass source through yeast use on high-pressure homogenizer. For this purpose, a design of experiments was needed so as to successfully analyse the test parameters for this energy improvement. The Design of Experiment (DOE) V.8 was used in creating the experimental run order and statistical analysis as well as the provision of extensive graphs that showcase the relationship between the input parameters and the output responses [38]. This also shows adoption of the response surface methodology which follows the Box-Behnken Design (BBD) with variables as shown in Table 1. [39]

$$Y = b_o + \sum b_i \chi_i + \sum b_{ii} \chi_{ii}^2 + \sum b_{ij} \chi_i \chi_j \quad (1)$$

4.8 Box-Behnken Design

Using the Response Surface Methodology (RSM), BBDs are response surface designs specially made to require coded values as -1, 0 and +1 with three levels. These are formed through combining two-level factorial designs with incomplete block designs. This procedure creates designs with desirable statistical properties but, most importantly, with only a fraction of the experiments required for a three-level factorial. Due to their levels being three, the quadratic model is therefore appropriate as blocking options are also offered for most of these designs [40]. This therefore in essence indicates that RSM is a set of mathematical and statistical techniques that are useful in the modelling, interpreting and predicting the response of interest to several input variables $\chi$ (level i to j). This is with aim of optimizing single or multiple response 'y' and this study, it is a single response (Protein Concentration; PC) measured in milligram/millilitre; (mg/ml) and the generated equation is shown in equation (1) above. The results of the experiment are as shown in shown in Table 2 according to the RSM design matrix, sorted by standard order.

The 2nd order polynomial model as indicated by equation (1) through stepwise regression was fitted and then applied on the response (Protein Concentration) measured in mg/ml. This was also used in generating Analysis of Variance (ANOVA) in Table 3 and response plot

## 5 Results and Discussion

Considering the data set provided for optimal results, which was needed to be tested in capturing the highest variability of response from minimum runs in terms of underlying factors being considered for energy improvement, the RSM provided 17 runs of experiment (Table 2).
While (Table 1) shows the parameter variables and their ranges of values in the coded form with their design level; pressure (30 – 90) MPa, temperature (15 – 25) °C and number of cycles (1 – 5).

**Table 1:**
RSM Showing design level and coded values against process variables

| Variables | -1 | 0 | 1 |
|---|---|---|---|
| **Pressure (MPa)** | 30 | 60 | 90 |
| **Temperature (°C)** | 15 | 20 | 25 |
| **Number of Cycles** | 1 | 3 | 5 |

Design of experiment has been applied here using the Box Behnken Design (BBD). 3 factors; temperature, pressure and number of cycles and a response of protein concentration were analysed with 17 runs of experiments as detailed above, their assigned ranges have been used in obtaining the experimental results.

**Table 2:**
Design Matrix

| | | Factor 1 | Factor 2 | Factor 3 |
|---|---|---|---|---|
| **Std** | **Run** | **A: Pressure** | **B: No. of Cycles** | **C: Temp** |
| | | **MPa** | **No.** | **°C** |
| **1** | 13 | 30 | 1 | 20 |
| **2** | 4 | 90 | 1 | 20 |
| **3** | 6 | 30 | 5 | 20 |
| **4** | 3 | 90 | 5 | 20 |
| **5** | 7 | 30 | 3 | 15 |
| **6** | 9 | 90 | 3 | 15 |
| **7** | 17 | 30 | 3 | 25 |
| **8** | 12 | 90 | 3 | 25 |
| **9** | 10 | 60 | 1 | 15 |
| **10** | 16 | 60 | 5 | 15 |
| **11** | 2 | 60 | 1 | 25 |
| **12** | 11 | 60 | 5 | 25 |
| **13** | 15 | 60 | 3 | 20 |
| **14** | 1 | 60 | 3 | 20 |

| | | | | |
|---|---|---|---|---|
| **15** | 14 | 60 | 3 | 20 |
| **16** | 8 | 60 | 3 | 20 |
| **17** | 5 | 60 | 3 | 20 |

5.1 Developed mathematical model for yeast cell wall rupture

The fit summary output indicates that for the response, the linear model are statistically recommended for further analysis as they have the maximum predicted and adjusted $R^2$ [41, 42]. The test of significance of the regression models, the test for significance on individual coefficients and the lack of fit test performed using the same statistical package for the response. Through selecting the step-wise regression method, the insignificant model terms can be automatically be eliminated. The resulting ANOVA table for the reduced linear model outline the analysis of variance for the response and illustrate the significant model terms. This also show the other adequacy measures $R^2$, adjusted $R^2$ and predicted $R^2$ as 0.886, 0.869 and 0.849 respectively. The entire adequacy measures are close to 1, which are in reasonable agreement and therefore indicate adequate models as well as the adequate being equal 20.9 for the concentration of protein yield, makes it an adequate model too [41, 42]. The adequate precision compares the range of the predicted value at the design points to the average prediction error. In this case, the values of adequate precision ratios are dramatically greater than 4. An adequate precision ratio above 4 indicates that the model is adequate; an indication of good model discrimination [41]. Fig 4a and 4b is the replica plot of predicted against the actual response and the residual of the normal plot of protein concentration response. An adequate model means that the reduced model has successfully passed all the required statistical tests and can be used to predict the responses or to optimize the process and so on. The final mathematical model linked to the response with regards to the coded factors and actual factors as determined by the software are respectively equations (2) and (3)

**Table 3:** ANOVA Table for Protein Concentration Reduced Linear Model

| | Sum of | | Mean | F | p-value | |
|---|---|---|---|---|---|---|
| Source | Squares | df | Square | Value | Prob > F | |
| Model | 0.34425 | 2 | 0.172125 | 54.14811 | < 0.0001 | Significant |
| A-Pressure | 0.32805 | 1 | 0.32805 | 103.1999 | < 0.0001 | |
| B-No. of cycles | 0.0162 | 1 | 0.0162 | 5.096292 | 0.0405 | |
| Residual | 0.044503 | 14 | 0.003179 | | | |
| Lack of Fit | 0.023483 | 10 | 0.002348 | 0.446869 | 0.8625 | not significant |
| Pure Error | 0.02102 | 4 | 0.005255 | | | |
| Cor Total | 0.388753 | 16 | | | | |
| $R^2 = 0.886$ | | | Adj $R^2 = 0.869$ | | | |
| Pred $R^2 = 0.849$ | | | Adeq- Precision = 20.9 | | | |

**Table 4:** Design Matrix showing the input and output (Response)

| | | Factor 1 | Factor 2 | Factor 3 | Response 1 |
|---|---|---|---|---|---|
| **Std** | Run | A:Pressure | B:No. of cycles | C:Temperature | Protein Conc. |
| | | MPa | No. | °C | mg/ml |
| **1** | 13 | 30 | 1 | 20 | 0.95 |
| **2** | 4 | 90 | 1 | 20 | 1.32 |
| **3** | 6 | 30 | 5 | 20 | 0.99 |
| **4** | 3 | 90 | 5 | 20 | 1.4 |
| **5** | 7 | 30 | 3 | 15 | 0.975 |
| **6** | 9 | 90 | 3 | 15 | 1.4 |
| **7** | 17 | 30 | 3 | 25 | 0.885 |
| **8** | 12 | 90 | 3 | 25 | 1.3 |
| **9** | 10 | 60 | 1 | 15 | 1.11 |
| **10** | 16 | 60 | 5 | 15 | 1.26 |
| **11** | 2 | 60 | 1 | 25 | 1.125 |
| **12** | 11 | 60 | 5 | 25 | 1.215 |
| **13** | 15 | 60 | 3 | 20 | 1.145 |
| **14** | 1 | 60 | 3 | 20 | 1.205 |
| **15** | 14 | 60 | 3 | 20 | 1.01 |
| **16** | 8 | 60 | 3 | 20 | 1.125 |
| **17** | 5 | 60 | 3 | 20 | 1.085 |

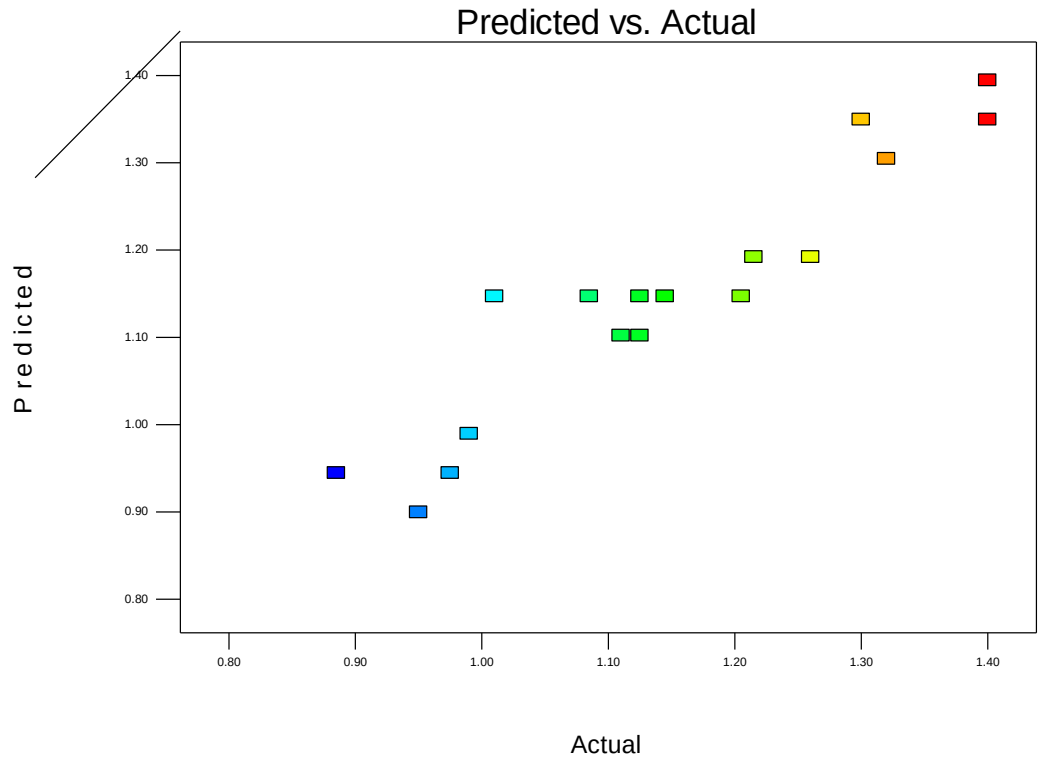


**Fig 4a:** plot of predicted against the actual response

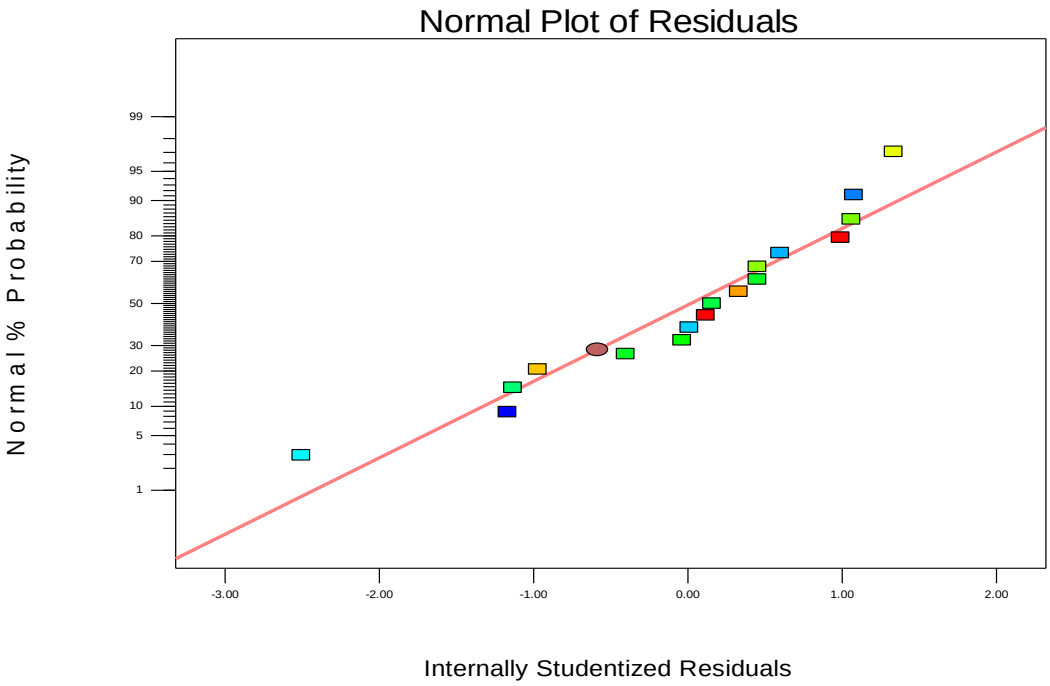


**Fig 4b:** Normal plot of residual protein concentration response

**Final Equation in Terms of Coded Factors:**

Protein Conc. = +1.15 +0.20* A +0.045* B (2)

**Final Equation in Terms of Actual Factors:**

Protein Conc. = +0.67456 +6.75000E-003Pressure +0.022500 * No. of cycles (3)

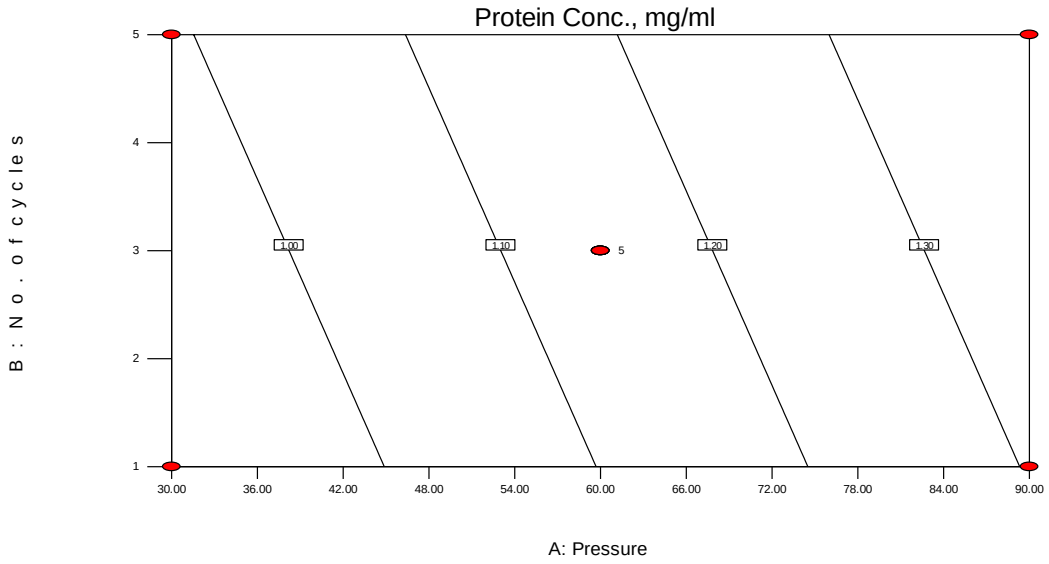


**Fig 4c:** Contours plot showing the effect of number of cycles and pressure on the yield of protein concentration (this shows zone with highest software-estimated protein concentration)

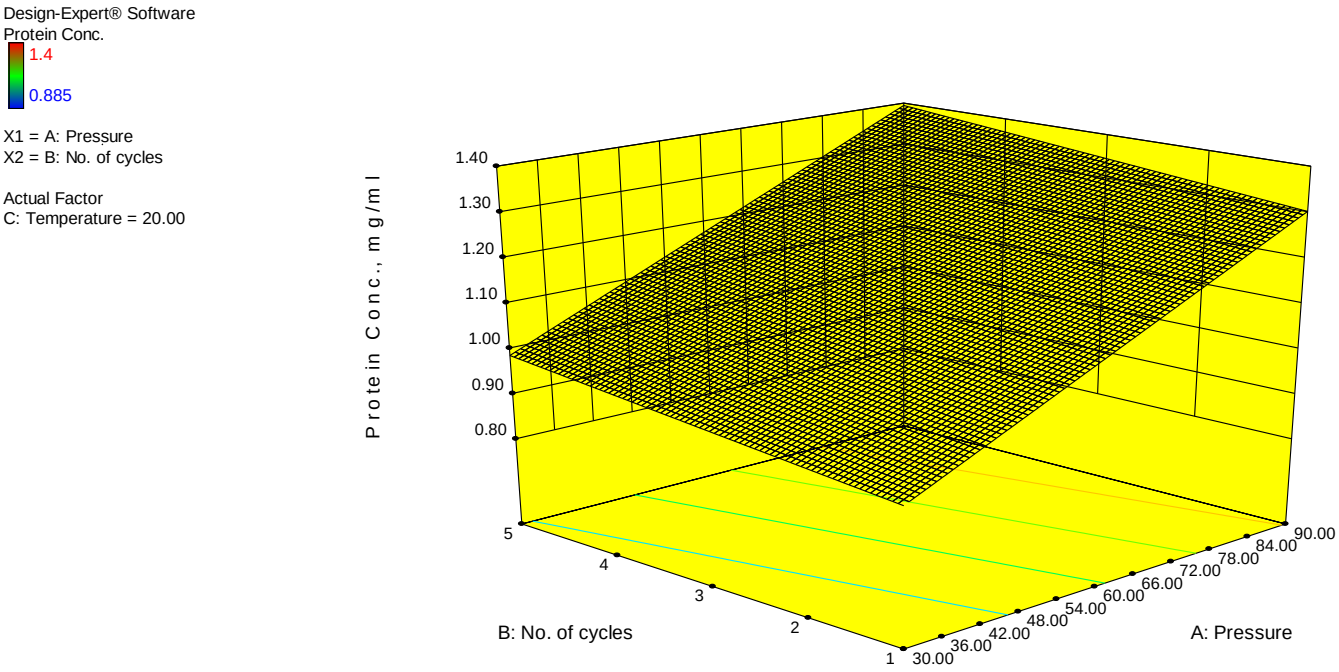


**Fig 4d:** Response surface plot of protein concentration yield (with actual factor temperature considered at 20 degree)

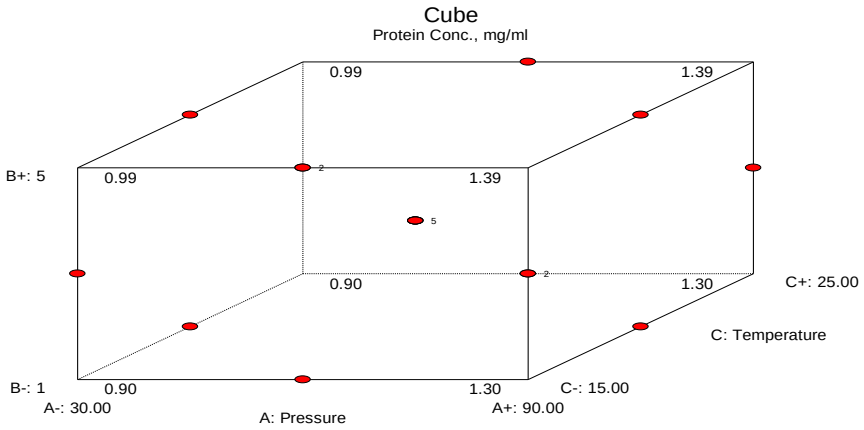


**Fig 4e:** showing the three factors A, B and C representing (Pressure, Temperature and Number of cycles) and their ranges in the cube

5.2 The Effect of Factors on the Response

The perturbation plot below shows how the protein concentration results as pressure having a significant effect in its production. Increase in the pressure results in high protein concentration yield; A while B; number of cycle shows no much rise in its effect to the yield of protein. Factor temperature is not having any effect on the yield at all and it is insignificant to the response variation and thus does not appear in such figure, while increasing A will in turn consistently improve the response. Factor B also affects the response following a linear behaviour.
Finally, the perturbation acts in such a way that the effect of the number of cycle be kept higher and that of pressure also, so as to improve the yield of protein concentration. This is therefore proven that temperature showed no effect in the improvement of energy through yeast homogenization in the HPH

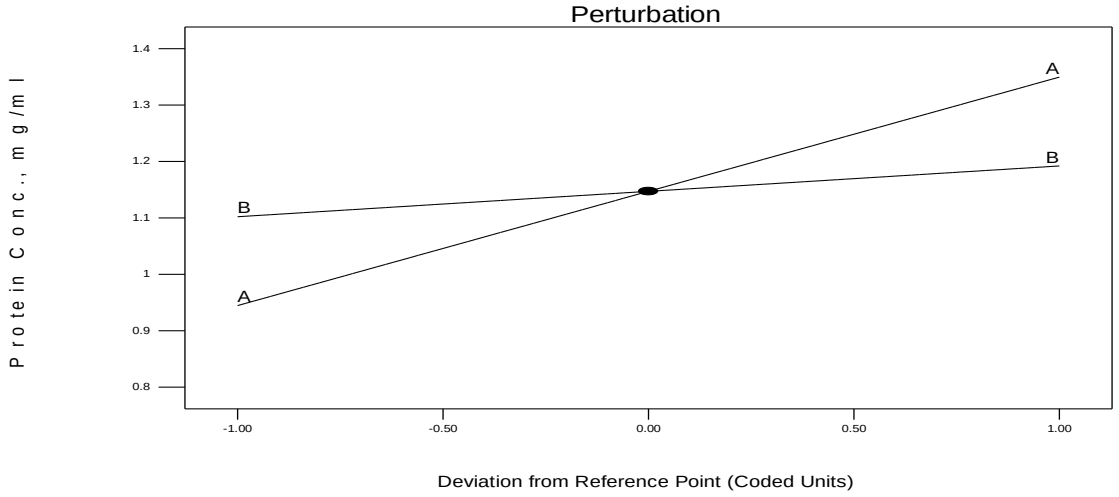


**Fig 4f:** Perturbation plot showing pressure increasing with the number of cycles as yeast is Homogenized

Contour graph is one of the plots that can be developed by design expert V8. This contour plot provides two-dimensional views where all points that have same response and are connected to produce contour line of constant response. (Fig 4c) is a contour plot for the effect of pressure and number of cycle on the protein concentration.
The effect of the two mentioned parameters on the response (protein concentration) can be presented in three dimensional in response surface plot as shown in (Fig. 4d). This plot provides clearer view of the surface.

5.3 Pressure effect

Pressure has been the most considerable factor in the use of HPH for cell disruption of materials during homogenization. The pressure exerted on the hand wheel during this process tends to close the gap between the inlet and outlet valve through shortening of the passageway for the homogenized yeast. Through the study of the causes of breakage and disruption in homogenizer, [14] highlighted homogenizer function as pump causing the pressure to build up to an unusually high value before the gap region and the gap width of the pump adjusted during flow by the operator which allows the operating pressure to be changed. The fluid at this point has reached the maximum velocity and then leaves this gap through the outlet in the direction of the impact ring. For multiple passes or number of cycles, the fluid is then recirculated to a continuous process of cell rupturing depending on the number of cycle. In a similar development, homogenizing *Escherichia coli (E.coli)* in the HPH, [43] studied pressure and verified it playing a major role in obtaining high yield of *nucleocapsid (NP)* protein. This was proven that highest production was achieved when the HPH was operated at high pressure.
In this study, pressure also played a dominant role in the homogenization of yeast and from the data above (Table 4), at pressure 90 MPa shown at runs **3**, 4, **9** and 12, the protein yield were **1.4 mg/ml**, 1.32 mg/ml, **1.4 mg/ml** and 1.3 mg/ml. These show highest yield of protein at the maximum pressure of 90 MPa while the lowest pressure at 30 MPa at runs 6, 7, 13 and **17** produced the least of protein yields of 0.99 mg/ml, 0.975 mg/ml, 0.95 mg/ml and **0.885 mg/ml** respectively (Table 4). This has further being straightened through the studies made by [44] that mechanical disruption process involves the actions of externally applied stress or pressure on the cells, this therefore shows that when the external pressure exceeds the internal pressure within the cell, disruption would have taken place [45]. Also, shear stress an influence on pressure, create a phenomenon which will be dependent on the relative velocity between the solid and the surrounding liquid whether the liquid is accelerating or decelerating and as a result a tensile stress is developed in the cell wall, wherein the hemisphere is created in front [14].

5.4 Temperature effect

Temperature as a factor showed no significance in this study. Though at slightly below room temperature of 15 °C, this yielded the maximum concentration of protein; 1.4 mg/ml on the highest applied pressure of 90 MPa, while at the highest of temperature of 25 °C of the yeast being homogenized, resulted in the least yield of protein concentration (0.885 mg/ml). This scenario shows that temperature had no effect on the yield of protein concentration as either high or low due to its inconsistency. Across the temperature range examined; 15 – 25 °C in which yeast has been treated in the high pressure homogenizer, it has critically been observed that protein concentration recorded has

shown different values with no consistency. From the given data and previous studies, have not been proven as to what has resulted to this. This may be entirely different when temperatures outside this range are considered but there has been no theory to prove this. In its own effect, temperature has played no role in this study but has played a role in pressure treatment of yeast in the HPH [46], and from previous studies [47, 48], the combined effect of heat and pressure processes are mainly based on empirical data on inactivation of microbes. This on the contrary, showed that the combined pressure and elevated temperature does not affect one specific site but have a number of targets affected [46].

### 5.5 Number of cycle effect

As previously highlighted in (Fig 4f) of the perturbation plot showing pressure increasing with the number of cycles as yeast is homogenized. Both factors of pressure and number of cycles acted in the same way in this study with much appreciable effect resulting from the applied pressure on the homogenized yeast. As the number of cycle sets in, it weakens the cells at the end of every cycle after passing the liquid yeast through the HPH, this therefore enables the cell wall to be fully broken down. After the first 2 – 3 passes, the entire cell wall of yeast would have been completely broken down for the release of protein. Though, this is considered possible through the application of shear stress on the HPH hand wheel as an influence to the exerted pressure at a high level of say 90 MPa. Samarasinghe et al. [49] have studied that nozzle size did not affect the degree of cell rupture, which then implies that the impact of shear exerted upon the cell walls from the nozzle walls was insignificant. This invariably means that more resistance are imparted from smaller nozzle in a forward movement thereby rendering more shear onto the cell walls as when compared to larger nozzle. The size increment is considered better through preventing clogging during real-life situation but as considered in this study, 2-stage homogenizing valve, size of nozzle was not the case but number of cycle improved the homogenization process wherein increased cell walls are broken down particularly when particle size distribution are considered.

## 6 Conclusion

The outcome of the experimental results shows a great potential of the novel biogas improvement through yeast use and cell wall breakage in HPH and as analysed using the design expert (DOE).

In fact, high pressure homogenization has proven to be an effective technique in the rupturing of yeast cell walls as when compared to other mechanical techniques for rupturing microbial products such as Micro fluidizer and Bead mill. An initial factorial design (design matrix) showed 17 runs of experiment for the 3 factors.

The following points have therefore been achieved from analysis;

- Conducted the homogenization process using the 2-Stage HPH GYB40-10S to obtain set results of the three factors (Temperature, Pressure and Number of cycles) and one response (Protein Concentration ) using the DOE in its analysis.
- In the build-up of protein yields, temperature never showed any effect on the production as shown in the DOE data information within the temperature range (15 -25 °C) considered.
- The diluted homogenized yeast has shown a significant results and this is assumed to also be possible with the undiluted yeast and whose results will be compared to that of the diluted yeast to determine how the protein yield has improved significantly.
- Pressure rise during the experimental work indicate that the viscosity of yeast being distorted and as a result tends to increase the protein yield, it therefore enable the cell wall to be disrupted to liberate the protein.

- Due to the roughness in the surface, at a certain velocity high enough, the streamline in the flow will no longer maintain the same shape and thereby deviates from the orderliness in the pattern of movement.
- The most significant parameters in this study were the pressure differential across and the number of cycles through the homogenizer. Though the maximum pressure tends to produce the highest of protein concentration but within the data set, this combined with the number of cycle yielded optimum result.

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