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Yeast: A Potential Biomass Substrate for the Production of Cleaner Energy (Biogas)

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Abstract

Yeast cell wall and its entire contents disruption treatments are required in the enhancement of protein and the overall biodegradability of the cell wall materials during homogenization process. Yeast as a cheap, good resource and easily available source of energy from biomass into biogas, it was used as a substrate for the cleaner energy production due to its richly and high level content of protein contained in it.
An initial study on the effects of high-pressure homogenizer mechanical pretreatment has been conducted in sequence as generated by the design matrix of the design of experiment (DOE) focusing on protein yields from bakers' yeast also known as *Saccharomyces cerevisiae* and in order to achieve the maximum yield of protein which in other words aid biogas production, the following optimum process parameters were set in. The yeast block was refrigerated at between 0 – 4 °C with fermentation at (0 – 24 h), a pH value of (5.3) maximum was used in the preparation of the buffer solution C. This was obtained through diluting solution B into A until the pH was attained (details as shown in the materials and methods section). Number of cycles (passes) of the soluble yeast were undergone to enable the yeast cell walls be broken down for the release of more protein and at temperature range (15 – 25 °C). The pressure for the compressed state during homogenization was set between (30 – 90 MPa). The results presented therefore showed the rates of protein released from the disruption through using the Design Expert Software V.8 in identifying the ideal conditions as set in the parameters.

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1. Introduction

The methods used for disrupting microbial cells can roughly be classified as mechanical (employing shear force, agitation with abrasives or freeze-thaw) and non-mechanical (treatment with acid or alkali, detergents as sodium dodecyl sulphate or Triton X-100, organic solvents as acetone, ethanol or toluene, osmotic shock and enzymatic digestion with lysozyme, glucanases and/or proteases) [1, 2]. Of the wide range of techniques available, not all are adequate for large-scale application but high-pressure homogenizer as a mechanical means of cell disruption have proven to be the most effective and efficient way for breaking down cell walls in baker's yeast at both laboratory and large scale use. Schutte and Kula [3] highlighted high-pressure homogenization technique as the most widely used method for process scale cell disruption for microbial cell wall (figure 1). [4] has reported that cell disruption must be achieved efficiently so as to ensure maximum release of product while at the same time the exposure to severe conditions in the homogenizer must be limited in order to minimise product denaturation and excessive

formation of small cell debris fragments. This can adversely affect subsequent downstream product recovery and purification [5]. Keshavarz-Moore et al. [6] in their work have also suggested cell disruption can occur by impaction but shamlou et al. [7] clarified it that this cannot be ruled as a mechanism of cell disruption as it is not the only mechanism by which by which cell breakage can occur.
Yeast (Saccharomyces cerevisiae) as a biomass substrate has turn to be a newly found name termed as the workhorse in the current biofuel industry due to its use in the production of ethanol as a results of the its composition [8]. Most widely used amongst microorganisms for ethanol fermentation is Saccharomyces cerevisiae; this is due to its ability to hydrolyse cane sucrose in to fermentable sugars and has the ability to grow under anaerobic conditions [9, 10], and because it requires certain amount of oxygen in synthesizing essential fatty acids and other compounds contained in it to form energy while others like Schizosachharomyces pombe shows additional advantages of high osmotic tolerance [11]. Beudeker et al. [12] in their study, considers baker's yeast (S. cerevisiae) as one of the most important biotechnological products due to its several industrial applications and as a commercial product has several formulations hence it can be grouped into two main types; compressed yeast (fresh yeast) in block (figure 2) form as well as the other kind; dried yeast. Petersson et al. [10] have elaborated on biomasses and their conversion to biogas and such, revealed that lignocellulosic materials contain cellulose and hemicellulose, and that they are bounded together by lignin.

In fact, the use of yeast has continuously improved over time from commercial needs to other sector such as for researches in the universities for energy production. Like other microorganisms, yeasts are surrounded by rigid cell walls that have to be disrupted by physical, chemical or mechanical methods in order to retain the valuable cell content [13]. Their cells (as shown in figure 3) are surrounded by a tough, rigid cell wall that represents 20 - 25% of the dry weight of the cell [14]. Yeast species; Saccharomyces cerevisiae and Candida albicans have been the main focus in terms of chemistry and yeast walls structure. The wall consists about 85 – 90% polysaccharide and 10 – 15% protein [15]. Nguyen et al. [15] have researched as part of their work polysaccharide component consisting of mixture of water-soluble mannan, alkali-soluble glucan, alkali-insoluble glucan and small amounts of chitin, while most of the protein is covalently linked to the mannan, hence it is described as mannaprotein. Other area yeast has shown its useful value is in the biorefinery process wherein yeast generated has as high as 50% [16], this therefore makes it much superior to other co-products from 1G bioethanol production which have been employed conventionally in cattle feeds. Yeast in essence, can maximize the utility, applicability and general animal nutrition qualities of low-protein feeds due to its high protein contents [17]. This in other words, Han and Liu [18] have substantiated that yeast may provide much of the nutritional value of Distiller's Dried Grains and Soluble (DDGS) as it has a favourable amino acid profile.

This paper therefore investigates the potential yeast biomass substrate provides for the production of cleaner energy as part of renewable energy source. The applicable technique here is based on yeast disruption in the HPH. The results are therefore presented through the design expert by identifying the ideal conditions for optimum protein release for biogas production.

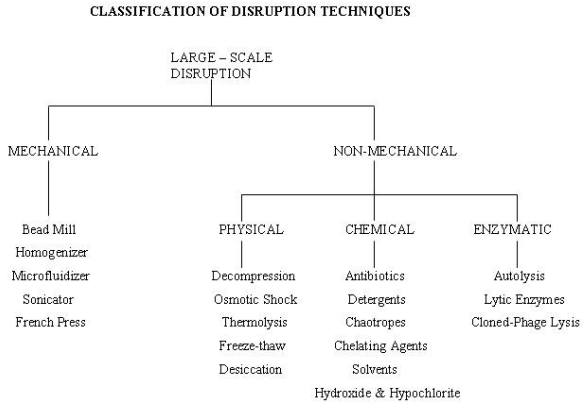

Figure 1: Cell disruption methods as modified from [19]

Figure 2: Block of Yeast

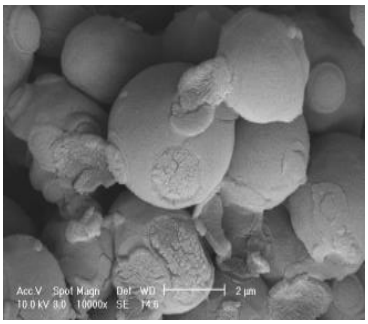

Figure 3: Yeast cells [20]

1.1. Yeast Importance

Yeast use has become a daily phenomenon in the industry and at research centres. Mostly common use is the bakers' yeast (Saccharomyces cerevisiae); it has reasonably well known genetic systems and act as a good host to produce desired intracellular material such as the protein. It can be used mechanically and non mechanically through the various cell disruption process. Yeast cell are regarded as hard to break as they normally needs multiple passes in achieving the high disruption rates required; this process on the ability to extract the valuable contents of the cells in a swift and efficient way [21]. Buijs et al. [22] have analyzed the usefulness of yeast as the yeast cell factory Saccharomyces cerevisiae can be turned into producer of higher alcohols (1-butanol and isobutanol), sesquiterpenes (farnesene and bisabolene), and fatty acid ethyl esters (biodiesel). In the advancement for biofuel, Steen et al. [23] engineered yeast to produce 1-butanol, but the titers were much lower than what has been obtained with E. coli (a few mg/L compared with g/L for E. coli). There is, however, much interest in commercial (iso-)butanol production using S. cerevisiae as a cell factory as demonstrated by a range of patents and patent applications by Butalco, Butamax Advanced Biofuels (a joint venture of BP and Dupont). Chen et al. [24] has been the first to report the production of Isobutanol from S. cerevisiae and overexpressed the endogenous genes of the mitochondrial valine biosynthesis pathway (ILV2, ILV5, and ILV3). Through this approach, they

increased the yield from 0.28mg/g to 3.86mg/g on glucose. Mathews et al. [17] have also reported that by generating yeast single cell protein (SCP) for use in cattle feed, the value chains associated with biofuels and cattle raising can be linked thereby resulting in expanded sugarcane cultivation for increased ethanol and food production. From this analysis McGinn et al. [25] have showed that the use of yeast S. Cerevisiae products as cattle feeds additives had no effects on methane emissions; or even reduced methane emissions.

Because they are widely used in the industry, their cells are in fact used as sources of food, vitamins and growth factors, this is due to the fact that they are cultured for cells themselves, for cell components and for the end products that they produce during the fermentation [26]. Intercellular material isolation needs the cell to be genetically engineered, so that what would normally be an intercellular product is excreted into the environment. This is normally through physical, chemical or enzymatic means to have this contents released into the surrounding medium [27]. yeast has been useful due to its commercial significance and value and it is having a long history as the organisms have been utilized in the fermentation of sugars in rice, wheat, barley in the production of alcoholic beverages as well as in the bakery industries, in these cases, the most commonly used type of yeast is the Saccharomyces cerevisiae referred to as the baker's yeast as mentioned previously [28], where its fermentation gives off ethanol and CO_2 through the sugar that is present in the flour, and most recently as a source of CO_2 which aid the production of biogas due to its high protein contents.

Figure 4: GYB40-10S 2-Stage Homogenizing Valves HPH

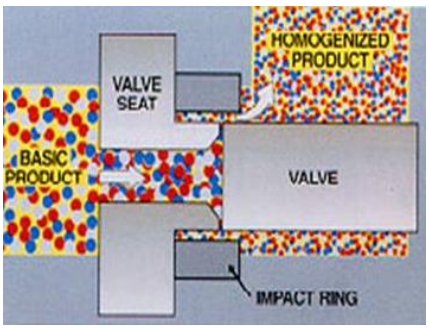

Figure 5: Inner components of HPH [34]

Figure 6: Homogenized Yeast

1.2. High Pressure Homogenizer (HPH)

High pressure homogenizer (HPH) is a highly skilled and advanced technological way of biogas production through disruption of bakers' yeast in the machine. It is extremely used in the emulsification, dispersion, mixing and processing of the products in various industrial sectors and one of the recent improvements in the design of the product is the ability to work under high pressure [29]. HPH machine is of different types and can deliver to the mangitude of up to 350 MPa but the GYB40-10S 2-stage Homogenizing Valves which is of concern in this study has 100 MPa pressures (maximum). This consists of a high-pressure positive-displacement piston-pump which forces the cell suspension via a value unit. The suspension is fed axially into the valve seat through the feeders' tank and then accelerates rapidly into a small gap between the valve head and valve seat. These then leaves gap and turns into a radial jet that stagnates on an impact ring before leaving the homogenizer at such an atmospheric pressure [30]. Depending on the pressure and number of passes, on exiting the machine, the suspension would have been fully homogenized breaking the cell walls to liberate the contents. In essences, successful recovery of the intercellular products like yeast assumes preservation of their contentsand the removal of the cell debris [31]. Uses of mechanical means have been regarded as the most appropriate and efficient way for industrial scale disruption and therefore allows the attainment of recovery at such a high standard. Realistically, they are prevented by high temperature, multiple passes with supplementary cooling as well as final products containing large quantity of cell debris [7, 31-33]. There are other parameters of interest to the machine during homogenization, such as; gap size, viscosity, turbulence, shear stress, impingement. Each and every of the mentioned parameter have a key role to play in the complete homogenization and liberation of the inner contents of the disrupted intercellular organisms. The final product of homgenized yeast is protein as shown in figure 6 and the high pressure homogenizer (GYB40-10S-2 Stage Homogenizing Valves HPH) and inner components of HPH (Valve seat, Impact ring and Valve head) are shown in (figures 4 and 5)

2. Materials and methods

2.1 Experimental set-up of high-pressure homogenizer

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 (HPH) and baker's yeast (figure 4) and (figure 2) respectively. The supplied baker's yeast was refrigerated for freshness originally and subsequently broken into beaker when ready to be used as it was as a block of fresh yeast, weighing 950g. Using the following composition; the solutions A, B and C were prepared.

2.2 Buffer solution

Solution A (0.1M KH2PO4 + 0.15M NaCl), this is equivalent of 1litre

13.6g of KH2PO4 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 K2HPO4 + 0.15M NaCl),
4.6g of K2HPO4 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.

2.3 Experimental technologies

The purpose of the experiment conducted was to determine the protein yield from homogenized yeast and analysis its potential as a biomass substrate through investigating the parameters; Pressure (P), Temperature (T) and Number of Cycle (N) under high pressure homogenizer using the Design Expert software V8. The yeast block sample, 950g was broken into beaker and mixed with solution C. The machine was turned on and fed with deionized water to clean and remove any impurities for about 20 – 30 minutes. The prepared soluble yeast was homogenized at 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 are shown below. 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 applied pressure closes the gap between the valve head and the seat and allows the yeast to flow out through the small opening. This clarifies the mechanism of cell rupture in terms of the rapid release of pressure as cells pass through the high-pressure homogenizer. The homogenized yeast is collected at the exit of the machine. 100 ml of undiluted homogenized yeast was measured into tubes and centrifugated for 13000 rpm over 60 minutes. Solution was separated from the debris and 500ml of the solution diluted with 2000ml of protein reagent for each of the samples. This was allowed to stay for half an hour before the protein value was 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 [35]

2.6. Design of Experiment (DOE)

This paper studies the different input parameters that are considered in the design of experiment analysis for the homogenized baker's yeast as a potential biomass substrate for a cleaner energy production. For this purpose, a design of experiments was needed so as to successfully analyse the test parameters for this energy improvement using the homogenized baker's yeast. 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 [36]. This also shows adoption of the response surface methodology which follows the Box-Behnken Design (BBD) with variables as shown in Table 2. [37]
The independent input variables and factor level are respectively (30, 60, 90) MPa for pressure A; (1, 3, and 5) as number of cycle B, and finally (15, 20, and 25) °C as temperature of the mixed yeast before homogenization. The experiment was planned based on a three level Box-Behnken design with full replication. Second order polynomials were fitted to the experiment data to obtain the regression equations and the sequential F-test, lack-of-fit test and other adequacy measures were used in selecting the best models. A stepwise regression method was used to fit the second order polynomial eq. (1) to the experimental data and to also identify the relevant model terms [38-40]. The same statistical software was

used to generate response plots. The values of the coefficients b0, bi, bii and bij can be calculated using regression analysis.

The probability > F (sometimes called p-value) of the model and of each term in the model can be computed by means of analysis of variance (ANOVA). If the prob. > F of the model and each term in the model does not exceed the level of significance (in this case $\alpha = 0.1$) then the model may be considered adequate within the confident interval of $(1- \alpha)$. An adequate model means that the reduced model has successfully passed all the required statistical tests and can then be used to predict the responses or to optimize the process [40]. Results are then used to run an optimization study using the numerical and graphical methods provided by Design-Expert in order to find out the best factors levels that under specific user-defined criteria will maximize the protein yield. The release protein is an indication of energy presence since this aid biogas production.

3. Results and Discussions

3.1 Model Estimation

The optimum combinations to be tested so as to capture the biggest variability in output (response) were provided by RSM. Table 2 shows the results of protein yield according to the RSM coded design matrix, sorted by standard order. The fit summary output indicates that the linear model is statistically significance for further analysis. A reduced linear model analysis was adopted for response resulting in the model terms of R2 = 0.886, adjusted R2 = 0.869, predicted R2 = 0.849, adequate precision = 20.9 for the protein. The values R2, adjusted R2 and Predicted R2 are close to 1 and so indicate the adopted model is adequate. The achieved adequate precision is >> 4, which indicates good model discrimination. The residuals are shown in figure 7a and 7b respectively for predicted against the actual response and residual protein concentration response. Since the internally studentized residuals are reasonably close to the normal probability diagonal, these figures indicate that the developed models are adequate and fit the data with normal distribution of probability.

Table1: RSM (BBD) process variables, design levels and coded values.

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

The analysis of variance (ANOVA) indicated that the number of cycles (passes) (B) and pressure (A) are more important factors to consider for protein yield as when compared with the third factor; temperature (C). The final mathematical model associated to the responses in terms of coded factors eqs. (2) and (3) determined by the software as shown below.

Final Equation in Terms of Coded Factors:

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

Final Equation in Terms of Actual Factors:

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

Table 2: Design Matrix and measured protein concentration yield.

Design Matrix					**Response**
Exp. No.	Run	Factor 1 A:Pressure (MPa)	Factor 2 B:cycles No.	Factor 3 C:Temperature (°C)	Response 1 Protein Conc. (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

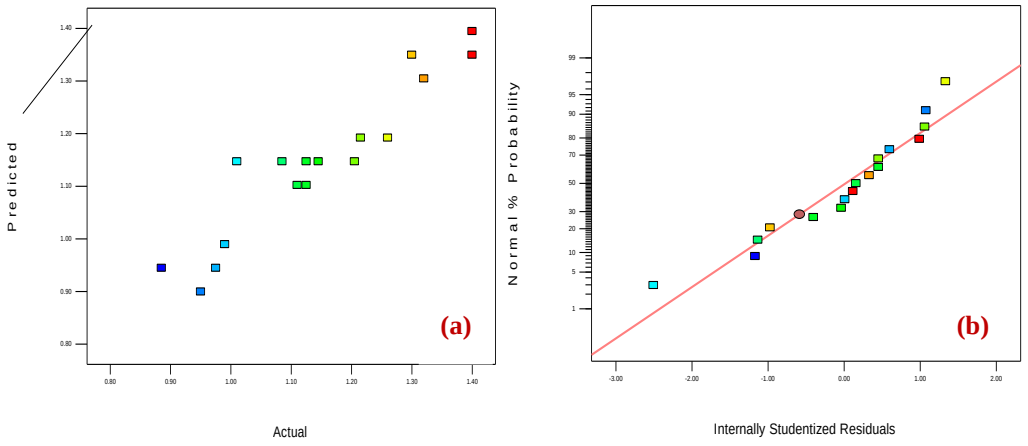

Figure 7: (a) Plots of predicted against the actual response and (b) normal residual protein concentration response.

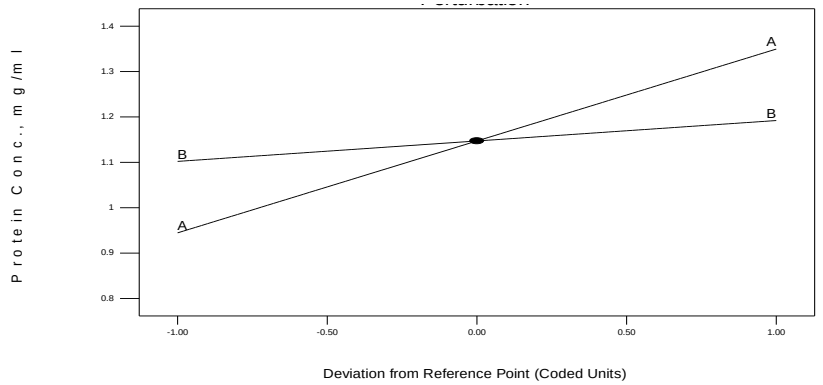

Figure 8: Perturbation plot showing the effect of process parameters on protein yield.

The perturbation plot in figure 8 shows that factors; A and B affect the protein volume response the same way. This showcase factor A affecting it in a steeper slope direction while B affects it in an almost horizontal direction. This therefore suggests the following i) increasing the pressure from 30 MPa to 60 MPa or decreasing it from 90 MPa to 60 MPa implies a positive effect on the protein concentration yield consistently; ii) desirably setting the number of cycle equals 1 and iii) A increase is beneficial to the steep forwardness the line have assumed which invariably have shown positive effect on the protein yield. This is necessary because complete destruction of the wall and the release of all intercellular components require destruction of the strength-providing components of the wall, that is; peptidoglycan in Gram-negative bacteria and glucan in yeast [41] and achieving this complete liberation will therefore requires increment in the applied pressure. Also increasing the number of cycle (passes) will enable the homogenized yeast to be elongated by stretching it to become slurry. Shynkaryk et al. [42] concluded that efficiency of High pressure homogenizer technique depends mainly, on the homogenizing pressure and the number of passes. Still on the issue of effects of pressure and number of cycles on protein yield, [27, 43] supported the view that by operating the homogenizer at higher pressures, it is possible to decrease number of cycles (passes) of the cell slurry through the homogenizer for a given degree of disruption.

However, three to five passages are usually required in the release of more than 90% of the protein but fewer passes are typically used for practical purposes, as the incremental amount of protein released by additional passages may not be economically justified [44]. The response surface so obtained is shown in figure 9.

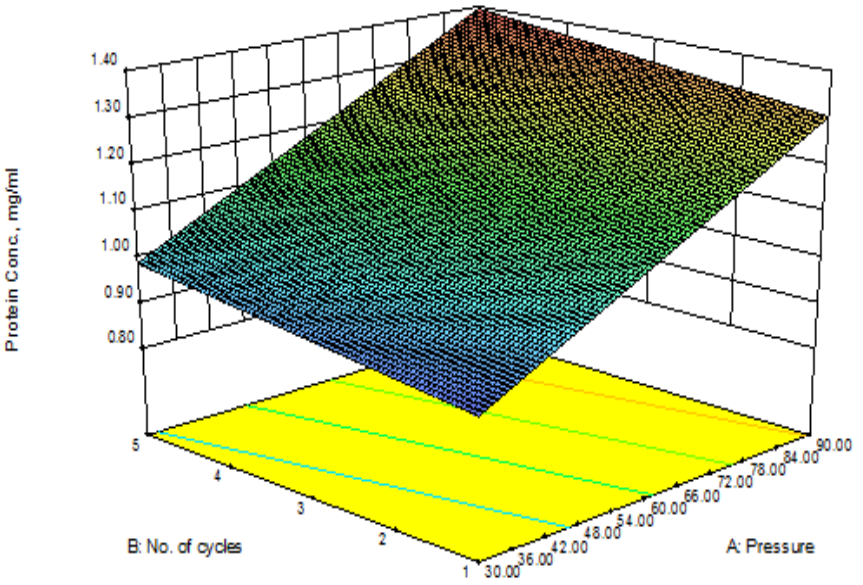

Figure 9: Response surface plot showing the effect of A and B on protein concentration rate at temperature = 20°C

Figure 10 is the contour graph showing the effect of pressure and number of cycle on protein concentration. In fact, the best results for protein concentration yields were achieved at 20 °C and 15 °C when using Pressure A = 90 MPa and Number of cycles = 5 and 3 respectively as the pretreatment settings during the homogenization (samples 4 and 6). Of the two samples, sample 6 tends to be better off due to the fact that more energy will be saved with reduced number of cycles (passes) and based on this fact, Uhlmann et al. [13] have concluded in their work through using the Avestin system high-pressure homogenizer wherein the first 100 MPa disruption pressure was chosen and 1-4 passes considered. This showed that one passage of cells is sufficient for cell disruption and no further shear stress needed to be applied because the protein yield in the supernatant in relation to the total protein content does not change significantly after the first passage. In this process, it could be very cost effective. However, [13] have regarded cell disruption mode not to have been fully elucidated as the performance is not always predicted but considers pressure, geometry of seat, valve, and impact ring as parameters that have influences on cell disruption and that can be adapted. While the velocity of cell suspension release and the number of passages can be modified.

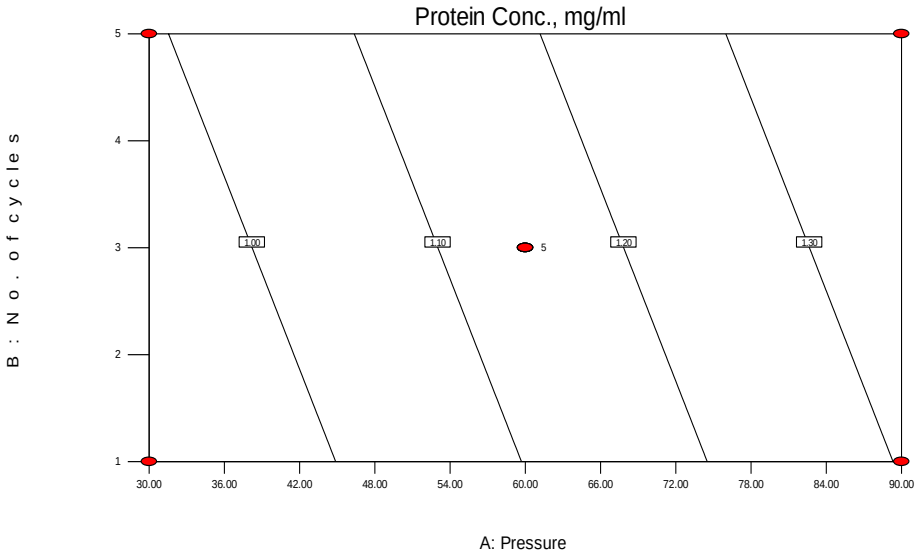

Figure 10: Contours plot showing the effect of A and B on protein concentration yield at C = 20 °C

3.2 Protein production

The design matrix generated through the RSM of three input factors of; pressure, number of cycle and temperature along with an output response of protein concentration yield. Across the examined input parameters applied in the conducted experiments, the results of protein yield are presented in table 2 (Design Matrix). An average of 1.147 mg/mL was produced from the 17 runs of experiment. The total protein yielded from the experiment amounted to 19.5 mg/mL. The results so obtained were generated from the six experimental samples conducted and average from the six experimental samples was recorded against each test results of the 17 runs of experiment conducted. The protein yield was obtained from the spectrophotometer by first recording the UV absorbance rate. The UV absorbance results for protein concentration yields were obtained from the protein standard curve which has been prepared from setting up Bradford assay using the bovine serum albumin (BSA). This works by having the curve calibrated and in the process enable to determine the exact protein concentration measure in a solution under investigation [45]. Runs of experiment between 13 and 17 were replicated in terms of the input parameter but resulted in different yield of protein as recorded in table 2. These are as a result of the operating conditions along with parameters considered, though temperature has shown no effect in the protein yield.

Conclusion

Based on the high pressure homogenizer used and within the limits of the homogenizing parameters as considered in this study, the following points can be concluded:

1. The homogenizing cost can be reduced by approximately 80% with acceptable proper-ties if the homogenizing condition are used

2. An experimental study of yeast biomass as a potential energy substrate in the high-pressure homogenizer using the Design Expert for analysis has been done.
3. Applied pressure of 90 MPa is an optimum input to obtain an excellent conversion of diluted yeast into protein concentration using the design expert software. This was considered optimal at 5 cycles and 3 cycles with an output of 1.4 mg/mL protein concentration. This is an indication that pressure did play a role in the protein production.
4. Temperature effect is of no significance in this study.
5. Design Expert is an accurate technique for analyzing the homogenizing process of the yeast conversion into protein concentration so as to obtain the best yield.
6. Yeast has shown to be a potential biomass substrate since its high protein contents aid in the production of biogas.

Therefore, current source for cleaner energy have shown that yeast as a biomass substrate will continue to be dominating in the plight for energy development amongst other biomasses. The reason being that it produces enough protein that can metabolize glucose and other sugars through anaerobic metabolism also known as fermentation and then releases CO_2 as a waste byproduct which also is useful in its own ways. From the experiments conducted, it has shown that when pressure is applied to the HPH, more of the cell wall that hold protein are broken down to liberate it. This therefore enables further fermentation of clean energy production to results.

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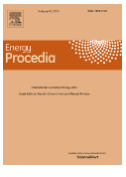

Biography

Leonard Ekpeni is a researcher and mechanical engineer into renewable and sustainable energy at Dublin City University, Ireland. His focus is on biomass conversion into biogas using mechanical technology; high-pressure homogenizer (HPH). His other areas of interest are; climate change, water pumps and wastewater technology.