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# Abstract

Anaerobic degradation of bioplastics is a controversial challenge. Size reduction is a must for degradation while it requires a significant amount of energy, which lowers the overall energy efficiency of the system. On the other hand, inoculum to substrate ratio has interaction effects in the process. The present work aimed to optimize these two parameters for the improvement of energy efficiency through response surface methodology. The central composite design procedure was implied. The levels of the experimental variables were 0.72, 4.3, 7.87 mm for particle size and 2, 3, and 4 for inoculum to substrate ratio. The input variable effects on biomethane yield were estimated, discussed, and then also optimized using the genetic algorithm. Moreover, energy balance analysis was done for the samples. The highest biomethane yield was found at the particle size of 4.3 mm and inoculum to substrate ratio of 4, which corresponds to 23% energy efficiency. Despite the high energy consumption for size reduction to less than 1 mm, more biomethane yield was not observed. Inoculum to substrate ratio showed more effect on biomethane yield than particle size.

**Keywords**: Biomass; Starch-based bioplastic; Optimization; Mechanical pretreatment; Biomethane yield

# 1. Introduction

Due to the environmental problems of petroleum-based plastics and the preservation of human food health, biodegradable polymers have received more attention since the 1970s (Nair et al., 2017). Concurrent with the advent of bioplastics, there were concerns about the biodegradation properties of them in nature. To this purpose, some research has been conducted on the biodegradability of these materials under various aerobic and anaerobic conditions. Among several testing methods which are available for monitoring the biodegradation of polymers (Shah et al., 2008), anaerobic degradation of bioplastics is still in its infancy (Bátori et al., 2018). As degradation of bioplastics in the soil is a lengthy process (Mohee et al., 2008), anaerobic digestion (AD) can be considered as a viable alternative to valorize them at the end of their life cycle. Since this bioenergy method is based on the final products of metabolism (mostly methane), it is believed that it shows precise results of actual biological degradation (Lucas et al., 2008).

Among the various types of biopolymers, those extracted directly from biomass (such as starch and cellulose) have received great attention. According to published reports, 21.3% of the world capacity of bioplastic production is allocated to starch compounds (Europeanbioplastic, 2019). Starch has a low price and good availability, in addition to being suitable for mixing with synthetic polymers (Platt, 2006). Along with microbial activity, it accelerates the degradation of polymer chain by producing pores and weakening them (Siracusa et al., 2008). For these reasons, starch-based bioplastic is a competitive raw material for AD in methane production. However it could be inferred from the literature in this field like Hirvonen (2019) and Vasmara & Marchetti (2016) that the main challenge of AD of starch-based bioplastics is possibly overcoming the low amount of produced methane. There are some pieces of evidence that they are not inherently biodegradable and starch in these bioplastics degrades partially (Quecholac-Piña et al., 2020). Another point to consider is that the rapid destruction of starch in AD may cause problems. An example of these problems has been demonstrated in the research of Russo et al. (2009) in which the pH of substances with more starch in their composition decreased to about 5 due to the accumulation of volatile fatty acids (VFA).

Temperature, carbon to nitrogen ratio, acidity, and the type of substrate and nutrients (macronutrients and trace elements) are the most important parameters that affect the AD (Zhang et al., 2014). Moreover, AD is more effective whenever the input raw materials are pre-treated before being loaded into the reactor. Pretreatments could increase the access of enzymes to substances and increase biogas production (Taherzadeh & Karimi, 2008). The appropriate range of these parameters can maintain the stability of the process. Chemical and thermal processing, as instance pretreatment for Polyhydroxybutyrate (PHB) could boost the hydrolysis of bioplastic, resulting in enhanced methane production (Venkiteshwaran et al., 2019).

Particle size (PS) reduction is considered as a desirable pretreatment for biological reactions. This pretreatment increases the release of soluble organic matter as well as the surface area of the solid particles, resulting in increased kinetics of degradation (Motte et al., 2013). Thus, digestion time, digester volume, and effluent sludge are reduced (Palmowski & Müller, 2000). However, conflicting results have been obtained so far. This inconsistency may be due to the release of inhibitory compounds as it was in the studies of Ryan et al. (2017) and Weiwei et al. (2016), bioplastic floatation on the inoculum (Yagi et al., 2012), and stocking of the materials due to static electricity (Yagi et al., 2012).

Yagi et al. (2012), held the view that the AD rate of biodegradable plastic depended on the form of the plastic material as well as the inoculum activity. Besides, Yagi et al. (2009) concluded that the rate of polylactic acid decomposition was not affected by the PS but by the microbial population. As a result, inoculum to substrate ratio (ISR) is another important parameter affecting the hydrolysis and consequently, production rate of methane (Moset et al., 2015). Rapid hydrolysis causes problems such as the accumulation of fatty acids, which is an inhibitor parameter for methanogens (Izumi et al., 2010). In order to reduce these problems, obtaining maximum degradation of polymer chains and turning them into biogas, the volatile solids (VS) content of the inoculum should be considered higher than VS content of the substrate (Zhang et al., 2018). For materials whose composition is relatively unknown or if there is a possibility of inhibitory issues, it is recommended to apply different ISRs. This ratio should be considered between 2 and 4 based on guideline suggested by (Holliger et al., 2016). A digester operating at very low ISRs (<2) creates toxic conditions for microorganisms (Raposo et al., 2006). More lag phase also is observed in these ratios, which means the microbial population needs more time to acclimatize and begin biodegradation (Moset et al., 2015). On the other hand, there is evidence of uncertainty in the results by using large amounts of inoculum (Angelidaki & Sanders, 2004). Therefore, ISR optimization is essential.

From the literature as mentioned above, it can be understood that various factors affect the biodegradation of bioplastics in anaerobic condition. Moreover, the interaction effects between parameters complicate the issue further. Using optimization models seems inevitable when maximum degradation rate/biogas production is a target. The application of statistical techniques in the development of biotic processes and optimization could result in enhanced product yields, suitable conformity of the response to objective criteria, and reduced operation time and cost (Maran et al., 2013). In this regard, the use of five different statistical models, including Gompertz, for optimizing the AD of Poly(3hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) have successfully been reported (Ryan et al., 2017).

As minimal literature is present on AD of bioplastics, particularly starch-based ones, it is necessary to assess the effective parameters and the kinetics in the degradation of starch

compounds, which are important constituents of this substrate. So far, some key parameters such as PS and ISR have not been documented clearly in previous AD studies of starch-based bioplastic. Also, their interactions complicate the issue. Pretreatments are cost and energy consuming, as well as high ISR might not be suitable. So optimization of these parameters is recommended. Based on the literature review, no research has been done on the modeling and optimization of PS and ISR using response surface methodology (RSM) and genetic algorithm (GA) to maximize the biomethane production of starch-based bioplastics as well as energy conversion efficiency. Therefore, this study aimed to (i) investigate the AD of commercial starch-based bioplastics taking into account PS and ISR, (ii) preparing an optimized model of biomethane production under such AD parameters.

### 2. Materials and methods

#### 2.1. Raw materials

Starch-based bioplastics of 0.45 mm thickness (Nooraste®), which is commonly being used and could be easily found in the market, was used for the experiments. They contain at least 60% of corn starch. They were crushed to pieces of different sizes by a mill (TOOS SHEKAN KHORASAN, IRAN). The maximum power requirement for the mill was 1200w. The pieces were then separated into different PSs by sieves NO. 30, 20, 8,  $\frac{1}{4}$  "and  $\frac{3}{8}$ " according to ASTM D1921-01. 0.72, 4.30, and 7.87 millimeters were the average PSs which were recovered on sieves NO. 30, 8 and  $\frac{1}{4}$ ".

## 2.2. Anaerobic digester

The required inoculum was collected from an active digester in the biogas laboratory of Ferdowsi University of Mashhad, which was in steady-state. The inoculum was first placed in a warm water bath at 37°C for 20 days to reduce its biogas production and be suitable for AD tests (Rosato, 2017). The biochemical methane potential (BMP) was conducted following the Holliger et al. (2016) procedure for anaerobic fermentation of organic materials. The eq.1 (Rosato, 2017) was used to achieve the ISR:2, ISR:3, ISR:4 while the total solid (TS) was adjusted to 5%.

$$ISR = \frac{V_{in}VS_{in}}{V_{sub}VS_{sub}}$$
(1)

In the above equation:  $V_{in}$  is the volume of inoculum,  $VS_{in}$  is the VS of inoculum based on wet weight,  $V_{sub}$  is the volume of substrate, and  $VS_{sub}$  is the VS of substrate based on wet weight.

The experiments were performed in 500 ml bottles with a working volume of 400 ml. Prior to pouring the media, the bottles were checked to be gas-tight. They connected then to two-liter gas collection bags through plastic tubes. Three digesters were filled with inoculum only (blank), and the average value of biomethane produced by them was subtracted from the amount of biomethane produced in the other samples to determine the substrate's contribution. On evaluating the quality of inoculum, microcrystalline cellulose from Merck Company in Germany was then used as positive control (Holliger et al., 2016). Before closing the lids of the digesters, carbon dioxide was purged for one minute on top of the solution to create anaerobic conditions. Fig. 1 shows the experimental setup.

#### 2.3. Compositional and analytical methods

During and after the experiment, various analyzes were performed according to the relevant standards. TS and VS of the substrates before and after the experiments were measured according to the American Standard for Public Health (APHA, 2005). Another fundamental analysis is the CHNS analysis, which was performed by the Thermo Finnigan device (FLASH EA 1112 SERIES). The amount of oxygen was calculated by subtraction. The data for this section is summarized in table 1.

The gas storage bag was used to measure the amount of daily produced biogas. The trapped biogas was measured by a lubricated syringe with a capacity of 60 mL (Raposo et al., 2012). An Einhorn fermentation-saccharometer containing 7 molar sodium hydroxide solution was used to obtain the percentage of biomethane. In this way, 5 mL of produced biogas was sucked in by the syringe and injected evenly into the solution inside the Einhorn. By this action, carbon dioxide dissolved in the solution and the remaining gas at the top of the container showed an acceptable approximate amount of biomethane (Stoddard, 2010). The ambient temperature of the laboratory during the experiment days had been measured by a mercury thermometer. Moreover, the daily air pressure of the location was adopted from the meteorology station in the university. These two data sets were then used to calculate the produced biomethane in the standard temperature and pressure (STP) according to Nielfa et al. (2015).

Measurements of biogas and biomethane continued for 26 days until biomethane production was less than 1% of cumulative biomethane production in three consecutive days (Holliger et al., 2016). The volume of the theoretical biomethane was also calculated according to the procedure defined by Buswell & Mueller (1952). Additionally, biodegradability was obtained as explained by Weiwei et al. (2016) (eq.2), which states

the feasibility of turning substrate into biomethane, carbon dioxide and biomass by microorganisms (Cho et al., 2011).

$$Biodegradability = \frac{V_s - V_B}{V_{Th}} \times 100$$
(2)

Where  $V_s$  is the cumulative biomethane production of the sample (mL CH<sub>4</sub>/g.VS),  $V_B$  is the cumulative biomethane production of the blank (mL CH<sub>4</sub>/g.VS) and  $V_{Th}$  is the theoretical biomethane production of the bioplastic (mL CH<sub>4</sub>/g.VS).

# 2.4. Statistical analysis

RSM is a set of mathematical and statistical techniques that are useful for modeling, interpreting and predicting the response of interest to several input variables with the aim of optimizing single or multiple responses (Tedesco et al., 2013). In the present study, the effects of the two independent variables (PS & ISR) on the response (biomethane production) has been investigated by employing a face-centered central composite design (CCD). This methodology was performed using the Design-Expert v.11.0.3 statistical software. The experimental design consisted of 27 experiments (three replicate for each specimen) with three center points. This design allowed not only the estimation of pure error and calculations of the response function at intermediate levels but also the estimation of the system performance at any experimental point within the studied range (Maran et al., 2013). The experimental variables of the independent factors were converted to coded variables. The general form of the predictive polynomial quadratic equation is described as:

$$y = \beta_0 + \sum_{i=1}^2 \beta_i x_i + \sum_{i=1}^2 \beta_{ii} x_i^2 + \sum_{i< j=2}^2 \beta_{ij} x_i x_j$$
(3)

Where  $\beta_0 \, \beta_i \, \beta_{ii}$  and  $\beta_{ij}$  are the constant, linear, secondary, and interaction effects of regression, respectively.  $x_i$  and  $x_j$  are the independent variables PS and ISR, respectively. y is the amount of biomethane production (mL/g.VS). By using a stepwise regression, the second-order polynomial model (eq.3) was fitted to the experimental data to acquire model terms. Then it was applied to the response, which in this case was the biomethane yields per gram of VS (mL/g.VS).

Afterward, analysis of variance (ANOVA) was conducted for fitting the mathematical model. The p-value of the model and each term in the model can be computed through ANOVA, so significant terms of the model were found and they were judged by the F-statistic calculated from the data. Besides, the experimental data was evaluated with various descriptive statistical analysis such as p-value, F-value, degrees of freedom (d*f*), the sum of squares (SS), the mean sum of squares (MSS), determination coefficient ( $\mathbb{R}^2$ ), adjusted determination of coefficient ( $\mathbb{R}^2_{adj}$ ) and correlation of coefficient ( $\mathbb{R}$ ) to reflect the statistical significance of the developed mathematical model. 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 (Tedesco et al., 2013). After fitting the data to the models, the generated data were used for plotting the response surface and contour plot.

In order to calculate the kinetics of the process, the modified Gompertz model (MGM) used for the best laboratory solutions (BLS) as follows:

$$B(t) = M_0 \cdot \exp\left\{-\exp\left[\frac{R_{max} e}{M_0} (\lambda - t) + 1\right]\right\}$$
(4)

Where B (t) is cumulative biomethane yield at digestion time t (mLCH<sub>4</sub>/g.VS),  $M_0$  is biomethane yield (mL/g.VS),  $R_{max}$  is maximum biomethane production rate (mL/g.VS/d),  $\lambda$  is the lag phase (day), and e is Euler constant (2.7183). MATLAB software was used for the curve fitting and estimation of the kinetic parameters of MGM. The R<sup>2</sup> and the root mean square error (RMSE) were calculated in order to evaluate the prediction accuracy of the models. On completion of optimization, optimal PSs for each ISR level were determined by the GA to maximize the biomethane yield of starch-based bioplastic. Then they compared with BLS. The final stage of the study comprised an energy balance evaluation at a laboratory scale.

#### 3. Result and discussion

## 3.1. Biomethane production

Across the period examined, the inoculum contribution to the overall biogas formation was 861 mL. Rapid initial biogas production was observed at all the tested ISR levels, which confirmed that the inoculum was well acclimated to bioplastic. The first phase of biodegradation, which involved the loss of physical characteristics such as discoloration (Muniyasamy et al., 2017), was performed in all treatments. The average biomethane productions, biodegradability, and their standard deviations have been shown in table 2. The biodegradability was assessed by measuring the amount of carbon mineralized from the starch-based bioplastic during the test (Gómez & Michel Jr, 2013). The samples produced biomethane between 135 mL CH4/g.VS and 250 mL CH4/g.VS during 26 days, in which the highest biomethane production was obtained for PS:4.3 and ISR:4 treatment. Biodegradability values varied between 42% and 78%. These values are considerably high when compared to literature such as (Gomez Barrantes, 2013) and are in line with values reported elsewhere (Cho et al., 2011). For example, Massardier-Nageotte and coworkers (2006) obtained 23% degradation (216 mL biogas/g) from 2 × 2 cm Mater-Bi® (MB) particles (starch and Polycaprolactone (PCL)). Similarly, the 1cm<sup>2</sup> MB particles produced only 33 mL CH<sub>4</sub>/g.VS in mesophilic monodigestion (Vasmara & Marchetti, 2016). In another study, two samples of starch-based film produced 113 mL CH<sub>4</sub>/g.VS and 69 mL CH<sub>4</sub>/g.VS in ISR:4 (Zhang et al., 2018).

However, another study (Mohee et al., 2008) investigated the biodegradability of MB (including 60% starch) at a size of 0.5-1 mm under landfill conditions, reported 245 mL CH<sub>4</sub> in 32 days which well degraded in comparison with the cellulose (248/8 mL). Also, Guo and co-workers (2011) reported that 58–62% biodegradation of starch/polyvinyl alcohol (PVA) based biopolymers are achievable under AD conditions, and wheat-based foam gave the highest ultimate CH<sub>4</sub> yield (293.7  $\pm$  6.7 mL/g.VS fed). Likewise, Hubackova et al. (2013) obtained 70% to 81% biodegradation for various starch/PCL compounds with glycerol.

### 3.2. Mean comparison with factorial design

The following part of this study moves on to determine whether any differences existed between the mean productions of samples. The results of the main effects and the interaction between the different levels of PS and ISR are demonstrated in Fig. 2. What stands out in this figure is a significant difference (P>0.05) of biomethane production between the different levels of PS and ISR from each other. The highest amount of biomethane was obtained from the PS:4.30 mm (Fig. 2 (a)). Therefore, it would not be necessary to reduce the size of bioplastic below 4.30 mm. This size had high enough surface area for the access of microorganisms. On the other hand, the higher the ISR value, the higher the biomethane production (Fig. 2 (b)). However, reactor construction restrictions might be a limiting factor. As can be seen in Fig. 2 (c), there were significant

interactions between the two parameters. Accordingly, the best results can be seen in the two treatments of PS:4.30, ISR:4 and PS:0.72, ISR:4 with biomethane yield of up to 249.89 and 246.00 mL of  $CH_4$ /g.VS (insignificance difference), respectively. These two treatments were considered as BLS.

#### 3.3.Modeling of biomethane production

As explained in the Materials and Methods section, the experimental design for optimizing biomethane rate was created using the CCD procedure. Four models were evaluated. The reduced cubic model had sufficient validity in biomethane modeling due to its  $R^2 = 0.99$  and non-significant lack of fit (LOF) factor. The  $R^2$  value of the model indicated that only 1% of the total variations were not explained by the model. The value of  $R^2_{adj}$  is very high and confirmed that the model was highly significant. The Predicted  $R^2$  ( $R^2_{pred}$ ) is in reasonable agreement with the  $R^2_{adj}$  since the difference is less than 0.2. So the results will be presented assuming a reduced cubic model. Table 3 shows the values of the coefficients of the reduced cubic regression model with their standard deviation.

Table 4 shows the ANOVA of the reduced cubic model to predict biomethane in terms of PS and ISR. The result of this table was obtained after removing the factors that were not significant. The ANOVA indicated that the main effects, the interaction and quadratic effects of the ISR, and other effects of the third degree have been significant at the 1% significance level. The model F-value of 575.03 revealed that the model was significant at p < 0.01. Furthermore, the LOF F-value of 1.2 and the associated p-value of 0.323 were insignificant due to relative pure error. This value means that there was a 32.3% chance that a "LOF F-value" this large could occur due to noise. Thus the eligibility of the model can be assured.

The result of model variance analysis is valid as long as the error distribution is normal, the treatment variance is identical, and there is no auto-correlation between the errors. Fig. 3 shows the results of the evaluation of these three hypotheses. Based on Fig. 3 (a, c), it can be inferred that the error distribution followed the normal distribution. Also, the assumption of the same distribution of variances is confirmed because the distribution of errors did not follow a specific trend (Fig. 3 (b)). In addition, the errors of the treatments did not have auto-correlation because their changes did not follow a particular pattern according to the number of treatments (Fig. 3 (d)). The validity of the ANOVA results was, therefore, reliable.

The experimental data of biomethane production were plotted against the values predicted by the reduced cubic regression model, as presented in Fig. 4. The agreement between the two data sets was close to the 45-degree line as the best fit line. Therefore, the predictions made by the regression model in the PS and ISR definition range can be reliable. As a result, RSM with a CCD could be efficiently applied to optimize biomethane yield in the AD of starch-based bioplastics.

What is evident in the perturbation plot (Fig. 5) is that PS affected the response in a concave way, while ISR affected linearly. It could be understood that decreasing the PS from 7.87mm to 4.3mm or increasing it from 0.72mm to about 4.3mm (to vertex) positively impact the biomethane yield. Also, increasing ISR is effective.

The percentage contributions (PC) on biomethane production were obtained for linear terms (PS and ISR) based on the SS (Maran et al., 2013). ISR had a more significant (almost double) share of biomethane production. It means that the microorganisms function was more effective than that of energy and cost intensive process of grinding.

More biological degradation was achieved as long as more microorganisms existed. To elaborate on this, the surface of the polymer was more broken and allowed microorganisms to had better access to the polymer. This process usually begins with microorganisms colonization on the surface (Tokiwa et al., 2009).

Fig. 6 demonstrate the biomethane response surface as a result of ISR changes from 2 to 4 and PS from about 0.7 to 8 mm using the extracted regression model. Increasing the ISR always raise the biomethane production, but this upward trend depends on the amount of PS. So that in the range of PS between 1 to 4 mm, it has the highest amount of biomethane production with increasing ISR. In ISR:2, some inhibition occurred for PS:0.72 mm, which appears to be due to the accumulation of fatty acids. As the ISR increases for 0.72, the slope of the contours decreases. This trend indicates that as the number of microorganisms increases, the intermediate products of digestion due to rapid hydrolysis become more rapidly convert to biomethane. Consequently, either the risk of accumulation or the inhibitory effects was reduced. However, the reduction in biomethane production compared to the PS:4.3 in ISR:4 can be justified by the floatation of particles on the surface of inoculum and/or stocking at the beginning of the experiment. It is worthy to mention that the floatation of PS:0.72 can be justified by Archimedes and Galilei principles. Floating of heavy objects arises as the interplay of the buoyancy and surface tension (Bormashenko, 2016). Although the density of bioplastic was equal in all of the samples, the surface area of smaller particles was more than the others. Also, due to surface tension, small objects could float on the media even though they are much denser than the media. Thus the floatation has occurred. It is suggested to reduce surface tension with appropriate approaches (i.e., using biosurfactants) for the application of smaller PSs.

Therefore, it can be claimed that reducing the PS to less than 1 mm did not have a significant effect on biomethane production. Relatively results were observed in which PCL powder did not seem to degrade better than which in the form of film (2×2 cm pieces), although it's available surface area was increased extremely (Massardier-Nageotte et al., 2006).

The microorganisms were not able to completely penetrate the large particles for cutting the chains inside the polymer, and probably depolymerization process was done incompletely. It means that extracellular enzymes have failed to convert long units into smaller molecules and prepare them for the next step of degradation by intracellular enzymes (Shah et al., 2008). That was clear from the low biomethane production for all treatments with PS:7.87 mm compared to the rest.

#### 3.4.*Kinetics study*

Having identified the BLS, the following section summarizes the goodness of fit and the results obtained from the kinetics study for them (table 5). Also, cumulative biomethane production using MGM has been predicted in Fig. 7. The usefulness of the Gompertz model, particularly for PHBV, was previously demonstrated. It could predict the biomethane yield considering surface area and PS (Ryan et al., 2017). In the current study, the correlation coefficients obtained were significant (p<0.05) except for PS:4.30, ISR:4 sample. This value suggests that the MGM was best fitting the experimental data. However, a more complex model is needed to predict biomethane for PS:4.30, ISR:4. Although there was a slight decrease in the yield of biomethane for BLS, the MGM ensured the increase in the biodegradability rate through AD for them. Nominal value of  $\lambda$  proof that the inoculum was well adopted for biodegradation of starch-based bioplastic.

The biomethane production rate for all treatments, except PS:7.87, was close to that of the cellulose until the third day. A similar result has been reported for plastarch during the first 7 days (Gómez & Michel Jr, 2013). On the other hand, biomethane was not produced For PS:0.72 and ISR:2 on days 7 and 8, which indicates a readily degradable material and an insufficient ISR in the reactor (Zhang et al., 2019). Also, it could be due to the high specific surface area (SSA) of these particles for microorganisms, which leads to rapid hydrolysis and accumulation of fatty acids (Ryan et al., 2017). Similar results were obtained for starch / PVA compounds with normal amylose between days 3th and 8th (Weiwei et al., 2016). Guo et al. (2011) reported that VFA production was high in days between 1 and 4, while there was no inhibition.

Although the PS:4.3, ISR:4 somewhat wasn't able to obtain the features of the biomethane production curves accurately ( $R^2=0.94$ ), it was favorable for avoiding the accumulation of VFAs.

#### 3.5. Optimization

The GA is a suitable method for biological processes among different optimization methods since many variables could be applied, and it is not dependent on the structure of the function for optimization (Saghouri et al., 2020). Table 6 shows the optimal PS values for the three levels of ISR using a GA to achieve the maximum amount of biomethane production. These values are 3.21, 3.46, and 2.64 mm for 2, 3, and 4 ISR levels, respectively. Less than these optimal values might cause temporary inhibition while more than them has little biomethane production. To confirm this, (Ryan et al., 2017) demonstrated that reducing PS less than optimal PS (0.8 mm) for PHBV had little effect on biomethane production and might cause temporary inhibition.

As the results confirm, the optimal PS value for different ISR values can vary. Additionally, the amount of biomethane produced at each optimal level was highly dependent on ISR. Increasing each ISR unit raised the biomethane production by approximately 20%. Comparing the results of GA optimization with the values of the BLS revealed that the optimal amount of expected production from the GA solution was about 3% higher than the BLS. On the other hand, due to the significant interactions, different PS values can be used. However, both are in common with the idea that high ISR will lead to more biomethane production.

### 3.6. Energy balance evaluation:

In the following section, specific energy productions from samples were calculated to find out how much energy gained. Firstly, the specific energy consumption was calculated and then compared with the heat value of produced biomethane (9.3 kWh/m<sup>3</sup> (del Real Olvera & Lopez-Lopez, 2012)) (eq.5) similar to other studies (Tedesco et al., 2020) with a modification ( $\eta_e$ ).

$$\eta_P = 1 - \frac{E_{pretreatment}}{E_{CH4} \times \eta_e} \tag{5}$$

In the above equation,  $\eta_p$  is the energy efficiency of the size reduction,  $E_{pretreatment}$  is the energy consumption of the mill,  $E_{CH4}$  is the heating value of biomethane produced by the target sample, and  $\eta_e$  is the conversion efficiency of engine for the electrical generation. The efficiency of engines for electricity generation varies from 30% to 40% (Lantz, 2012). The middle efficiency has been taken into calculations (35%). For the aim of future extrapolation, the results were calculated based on MJ energy per kg of VS (MJ/kg.VS). The energy consumption for size reduction was estimated by considering the mill

maximum power (1200w) multiplied by the usage duration of the mill (30s). At each use, 100 grams of bioplastics were poured into the mill. Then they were shaken into the stack of sieves. By this action, on average 13 grams PS:0.72, 19 grams PS:4.30, and 25 grams PS:7.87 were recovered. This process continued until the desired value was reached. Thus, the energy consumption for the 0.72, 4.30, and 7.87 particles was 3.29, 2.25, and 1.71 MJ/kg.VS, respectively. The effective energy efficiency has been computed for all combinations of PS and ISR and plotted as a bar chart (fig. 8), showing that under certain circumstances the results were negative. The best Specific energy production was for PS:4.3, ISR:4 with the value of 2.92 MJ/kg.VS, which about 23% energy gained (0.67 MJ/kg.VS). Although PS:0.72 produced remarkable biomethane in ISR:4, considerable energy loss had occurred in all PS:0.72 samples. This dissipation, indicating that the size reduction to 0.72 is highly energy-consuming. Though PS:7.87 had minimal productions in all ISR levels, they were valuable in terms of energy efficiency. This size had 20% energy efficiency in ISR:4, which was close to the best one. So if the net energy production be important, PS:7.87 could be used instead of PS:4.3. It should be mentioned that the extra energy derived from inoculum contribution hasn't been counted in these calculations. Also, most of the literature implied that full-scale studies minimize the input energy considerably (Atelge et al., 2020) by using more efficient facilities. Therefore the energy results could be improved.

### 4. CONCLUSION

The developed model using RSM was adequate and fitted the data. The best result of biomethane production was achieved at the treatment of PS:4.3, ISR:4 (249.89  $\pm$  2.36 mL CH<sub>4</sub>/g.VS) which gained 23% energy efficiency. This value is high when compared to previous research. The obtained results outlined that ISR played an influential role as a

biotic condition in the AD of the starch-based bioplastics. By optimization of the PS, biomethane production can be increased. The optimal PS for biomethane yields were identified at each ISR, which were 3.21, 3.46, and 2.64 mm for ISR:2, ISR:3, ISR:4, respectively. These optimal values produced 3% higher biomethane than BLS. It should be considered some chemical and physical properties of bioplastics such as surface area, molecular weight, and chemical structure for further research.

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Substrate & Inoculum

Figure 1. Experimental setup



Figure 2. The effect of (a) PS, (b) ISR and their (c) interaction on biomethane yield



Figure 3. Evaluation the validity of the regression model extracted according to the assumptions that the ANOVA is valid



Figure 4. The result of evaluating the agreements between experimental biomethane values and their predicted values by the reduced cubic model



Deviation from Reference Point (Coded Units)

Figure 5. The perturbation plot





Figure 7. The predicted values of the MGM for BLS, PS:4.30, ISR:4 (a) and PS:0.72, ISR:4 (b) with lower bound (LB) and upper bound (UB).



	TS (%)	VS (%TS)	C (%)	O (%)	H (%)	N (%)
Starch-based bioplastic	97.78	86.16	53.83	37.83	7.81	0.53
Inoculum	4	71	-	-	-	-

 Table 1. Composition of raw materials

Exp. No.	PS (mm)	ISR	biomethane production (mL CH4/g.VS)	Biodegradability (%)
1	0.72	2	157±2.11	$48.7 \pm 0.9$
2	0.72	3	185.71±3.10	57.7±0.9
3	0.72	4	$246 \pm 3.78$	76.4±1
4	4.3	2	165.57±2.57	$51.4{\pm}0.7$
5	4.3	3	196.99±2.43	$61.2{\pm}0.7$
6	4.3	4	249.89±2.36	$77.6 \pm 0.7$
7	7.87	2	$134.78 \pm 1.98$	$41.8 \pm 0.6$
8	7.87	3	161.13±1.65	$50{\pm}05$
9	7.87	4	$182.18 \pm 3.10$	56.6±0.9

Table 2. Design matrix and measured biomethane yield

Term	Coefficient	Std Coefficient
$\beta_0$	219.50	20.30
$\beta_1$	-17.90	3.90
$\beta_2$	-71.70	14.30
$\beta_{22}$	19.04	2.38
$\beta_{12}$	18.43	2.77
$eta_{112}$	-0.68	0.03
$\beta_{122}$	-2.57	0.45

Table 3. The coefficients of the reduced cubic regression model with their standard deviation

 $\frac{\beta_{122}}{y = \beta_0 + \beta_1 PS + \beta_2 ISR + \beta_{22} ISR^2 + \beta_{12} PS \times ISR + \beta_{112} PS^2 \times ISR + \beta_{122} PS \times ISR^2}$ 

Source of Variation	SS	df	MS	F-value	p-value
Model	36915.2	6	6152.50	575.03	0.00
A-PS	6109.2	1	6109.20	570.97	0.00
B-ISR	24356.5	1	24356.50	2276.4	0.00
$B^2$	380.4	1	380.40	35.56	0.00
AB	1297	1	1297.00	121.22	0.00
$A^2B$	4432.6	1	4432.60	414.28	0.00
$AB^2$	339.5	1	339.50	31.73	0.00
Error	214	19	10.70		
Lack-of-Fit	25.3	1	12.6	1.2	0.323
Pure Error	188.7	18	10.5		
Cor Total	37129.2	26			

Table 4. Results of ANOVA of the reduced cubic model for the prediction of biomethane yield

 $R^2 = 0.99; R^2_{adj} = 0.99; R^2_{pred} = 0.99$ 

	PS:4.30, ISR:4	PS:0.72, ISR:4
RMSE	16.08	4.09
$\mathbb{R}^2$	0.94	0.96
$M_{0}$	238.8	241.1
$R_{max}$	28	24.39
λ	9.69E-09	8.74E-07

Table 5. Goodness of fit & MGM parameters

	PS (mm)	ISR	Biomethane (mL/g VS)
	3.21	2	166.34
GA solutions	3.46	3	200.29
	2.64	4	256.39
BLS	4.30	4	249.89
	0.72	4	246.00

Table 6. Optimal Biomethane Production Values Presented by GA and Comparison with BLS



Inoculum

	PS (mm)	ISR	Biomethane (mL/g VS)
GA solutions	3.21	2	166.34
	3.46	3	200.29
	2.64	4	256.39
Best experimental	4.30	4	249.89
	0.72	4	246.00