Optimization of Cobalt Nanoparticles for Biogas Enhancement from Green Algae Using Response Surface Methodology

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Abstract
Organic matter may be converted to energy through various methods, but the most preferable one is the Anaerobic Digestion (AD), specifically for biogas production. In sustainable bioenergy production, it can undoubtedly be called one of the most widely used methods from the various feedstock. Over the past years, algae waste has become an increasingly acute environmental problem but luckily it can be used as feedstock to produce bioenergy. In order to improve the energy productivity of green algae, this study is focused on the introduction of cobalt (Co) nanoparticles (NPs) in the AD process. The concentration of Co NPs was optimized using response surface methodology (RSM). Mesophilic temperature range (25–45 °C), initial pH (5–9) and Co NPs dosage (0.5–2 mg/L) were selected as the independent variables for RSM. The results indicated that at optimized values (Co NPs concentration = 1 mg/L, initial pH = 7, and digestion temperature = 35 °C) produced the highest biogas yield of 298 ml. An experiment was carried out at optimized conditions to explore the effect on biogas production. The results showed that Co NPs had a positive influence on biogas yield. The low concentrations achieved higher biogas production as compared to higher ones. A maximum biogas yield of 678 mL is achieved by Co NPs (1 mg/L). AD performance was further evaluated by the modified Gompertz model. Different kinetic parameters were calculated. The values of the performance indicators confirmed that the mathematical model fitted well with experimental data.

Keywords
Anaerobic Digestion, biogas, cobalt nanoparticles, mathematical kinetic models

1 Introduction
The significant increase in the energy crisis has resulted in acute energy demand worldwide [1, 2]. The conventional sources are limited and depleting over time. With an increasing population and the human race for industrialization, stress on these non-renewable resources is increasing. On average, about 81 million people are added to the globe each year which makes the situation of meeting energy demand more challenging. In addition, fossil fuels are non-renewable and not environment-friendly in nature. Fossil fuels currently contribute more than 88% of global energy production but it is accompanied by the release of excessive CO2 to the environment. Excessive CO2 release is a enemy
of our environment as CO₂, due to its high RF (radiative forcing) value, has tendency to make earth much warmer as compare to other pollutants [3]. Due to this increased temperature of earth, weather becomes intense each year and people tend to use more energy to keep themselves at an optimum temperature. In recent years, some areas have experienced highest temperature in their history whereas some experienced deadly frozen conditions that were never experienced before. Due to these weather conditions, energy consumption, water consumption and consumption of other natural resources is increased. Based on this, it can be predicted that if temperature of earth will keep on increasing, energy demands will drastically increase [3]. Therefore, a great need arises to explore eco-friendly renewable sources to fulfill the energy demand [4]. One such source is energy harvesting via biomass.

Organic feedstock can be used to produce biofuels [3]. Organic matter may be converted to energy through various methods, but the most preferable one is the Anaerobic Digestion (AD), specifically for biogas production [5, 6]. AD is considered a common and valuable solution to process organic waste and ferment it to produce methane and hydrogen [7]. AD finds its implications of waste treatment on a broad category of waste including sludge, wastewater and municipal waste [8]. It is also mentioned among widely considered methods for conversion of complex waste to biogas [9, 10]. Additionally, applications of AD in the treatment of animal manure [11], energy crops [12], organic food waste [13], microalgae [14] and agricultural residues [15] make it stand among other methods.

In AD during the process of degradation and transformation of organic waste into hydrogen, methane, and carbon dioxide various anaerobic microorganisms are utilized. However, for their reproduction and growth, they need some essential nutrients. This complex process comprises of four stages as follows [16]. The very first stage is the hydrolysis stage. In this process, simple soluble monomers or dimers are produced by decomposing complex insoluble macromolecular organic aggregates. Second is the fermentation stage, it is the conversion process of small molecule products of the hydrolysis process into volatile fatty acids and nutrients with the action of microorganisms. Next is the hydrogen and acetic acid production stage, and it is by far the most complex one. In this stage, acetic acid and hydrogen is produced by the action of microorganisms on the products of the fermentation stage, such as alcohol, volatile fatty acid, lattice acid, etc. In the methanogenesis stage, there exist two methods of methane production which involve utilizing various activities of microbial groups [16]. Thus, formed methane, which significantly vary in quality based on a few factors such as biomass composition, additives, selection of conversion process and precursors. Typically, the composition of biogas is specified by methane, and carbon dioxide contributing 50–75% and 25–45%, respectively. A minute amount of other gasses can be there usually of calorific values 21–24 MJ/m³ [17].

Algae is a potential organic waste for the production of biogas and biohydrogen [18]. It can be seen from related literature a wide range of microorganisms can be utilized for hydrogen fermentation with a variety of pure monosaccharides, however, the use of pure monosaccharides makes it less economical [19]. Therefore, an effective way of reducing the production cost of biological hydrogen would be to anaerobically ferment the hydrolysate of algae rich in monosaccharides which are available by many microorganisms. Hence, there exists a broad application prospect for the study of algae as raw material for anaerobic fermentation. Enteromorpha is freshwater algae [20] containing cellulose, carbohydrates, and protein in huge amounts. Among other economic seaweed, Enteromorpha is considered an important one. It grows and reproduces rapidly, Enteromorpha has shown a rapid rise in growth in recent years due to seawater eutrophication aggravation along with the other favorable factors for its growth including climate warming. Its growth rate became so fast that it was named as “green tide” which shocked the entire world [21]. In the last about one and a half-decade, various green tidal natural disasters have been the fate of China. The current situation of energy shortage is closely followed in this study to explore the efficient pre-treatment experimental method of green tide algae Enteromorpha, which laid the foundation for large-scale use of algae to prepare bioenergy [22].

Nanotechnology is considered one of the most significant advancements in science and technology over the last few decades. However, the contemporary use of nanomaterials in bioenergy production is very deficient. Nanomaterials (NMs) are materials having one or more dimensions smaller than 100 nm [23]. This resulted in a much high surface area of the material just because of the size. A spherical nanoparticle (NP) of 1 nm diameter will have approximately 100% of its atoms on the surface. Whereas a NP having a diameter of 10 nm would have only 15% of its atoms on the surface. It would be expected from a particle having a higher surface area to be more reactive than the same mass of material consisting of
larger particles, as chemical reactions normally take place at surfaces. Literature is evident that nanotechnology is among the fast-growing field with respect to its application in a wide range of technologies [16, 24]. A few studies have reported the effect of nanoparticles and nanomaterials on the modification of feedstock. Especially, introducing NPs to the AD process has shown a significant impact on biogas growth [25]. Studies reveal that the addition of NPs determines the concentration of nutrients in the feedstock which has a direct impact on the production of biogas. A high concentration of nutrients results in inhibition, while a low or a medium concentration results in the enhancement of biogas production. Trace metals are essential for methanogenic bacteria growth in an AD reactor [26–28]. Metals nutrients such as iron, cobalt, nickel, etc., are found to significantly influence the AD process [29, 30]. Trace metals worked as an electron donor in an Anaerobic Digestion process. They increase the total consumption of hydrogen methanogens and activity. Trace metals release ions into and contribute to the production of key enzymes [26]. They can also optimize the microbial population, change the hydrolysis fermentation types and stimulate the acetic acid content [31].

Zero-valent iron has been widely employed for the treatment of various kinds of waste. The literature showed that during the AD process, it releases electrons for methanogenesis, resulting in biogas augmentation. Nanoscaled Zero-Valent Iron has a high surface-to-volume ratio. This characteristic increased the chemical reaction sites and produced an affirmative influence on the AD. Abdelsalam et al. [32] studied the effect of Ni NPs, Co NPs, and Nanoszerovalent Iron on biogas production from cattle manure. The results showed that all metal NPs increased biogas production.

Response surface methodology (RSM) is proposed by George E. P. Box and K. B. Wilson [21, 33]. This tool can be utilized effectively to improve and optimize unknown systems or processes through statistical analysis and mathematical methods. In RSM, a fitting curve was established that was based on the statistical model of information as a response to the surface design. The generated scientific map described the relation the variable and response [34]. RSM is a widely accepted technique for the design of the experiments and many researchers have reported optimization studies based on RSM. Jeong [35] used the RSM technique to optimize Levulinic Acid optimization from marine algae. Neifar et al. [36] was interested in the optimization study of enzymatic saccharification of Chaetomorpha linum to enhance the production of bioethanol. Similarly, Raina et al. [37] used RSM to maximize the production of bioethanol from enzymatic hydrolysis of deodor sawdust.

The purpose of this study was the exploration of the effect of Co NPs on the hydrolysis of green algae (Enteromorpha) and find out the variation in biogas production after treatment. In order to find out the most feasible and optimum concentration of NPs of Co, the RSM method is applied. Furthermore, to observe the relationship between the rate of the AD process and Co NPs previously established predictive models to determine the kinetics of the reaction. Mathematical models can be used to explain effects of the various components and to perform different behavioral predictions. Many studies use mathematical models to study the kinetic parameters, such as the biogas production potential, the maximum biogas production rate and the biogas production delay time. Between the many kinetic models for the gas production, are well known Gompertz and logistic models. Therefore, these models were fitted for microalgal biomass Anaerobic Digestion. These kinetic models are used to describe the data and estimate the biogas production potential from models’ parameters.

2 Materials and methods
2.1 Raw material
Wenchang Sewage Treatment Plant in Harbin, China was used to extract the anaerobic sludge used for the test runs. As the extracted sludge was aerobic, cultivation of anaerobic sludge was required. About 1 to 2 weeks were dedicated to proper aeration of the sludge. During the culture, three nutrients were introduced named NH$_4$Cl, KH$_2$PO$_4$ and glucose with a ratio of 5:1:300. After substantial settlement, the color of the sludge was brown. [38, 39]. Enteromorpha was used in this experiment that was courtesy of the Institute of Hydrobiology, Chinese Academy of Sciences. It was dried in a box and resulted morphology is depicted in Fig. 1. In Enteromorpha it contains 13.20% protein, 1.06% fat and 21.77% ash [40]. Spherical shape Co NPs (average size <100 nm) were purchased from China Metallurgical Research Institute, Beijing, China. Suspension of Co NPs was prepared for the purpose to reduced agglomeration by adding distilled water containing sodium dodecylbenzene sulfonate (SDS) 0.1 mM [41, 42].

2.2 RSM parameter design and experimental setup
For the experimental setup, 500 ml lab bottles sealed with rubber were used as a biogas digester. These bottles were
filled with 60 ml sludge and 20 g *Enteromorpha*. The amounts were selected based on the previous study conducted by us using response surface methodology (RSM) for optimization of *Enteromorpha* and anaerobic sludge digestion [21].

To initiate the AD process, N$_2$ gas was purged into the bottles for 300 seconds [43, 44]. In this experiment thermostatic steam bath vibrating (THZ-92A) was used, and samples were observed in batch mode for about 168 hours. After that, Design Expert Software (11.0.0) was used for constructing the design of experiments with central composite design. The input parameters for the DOE were Temperature (A), pH (B), and concentration of CO NPs (C), whereas biogas production was selected as the response parameter, see Table 1. The effect of these parameters was investigated by the experiment during the intermittent period. Frequency of each experiment was equal to three, and to minimize experimental errors solid-liquid ratio of deionized water and *Enteromorpha* powder was fixed.

### 2.3 AD process kinetic models

The logistic function model (Eq. (2)) [45, 46] and the modified Gompertz model (Eq. (1)) [47] was employed to evaluate the kinetic performance of the process of AD. The experimental data was fitted to the two models by OriginPro (9.5.1.195) software, and to complete the whole calculation the iterative method using the Levenberg-Marquardt algorithm was used. Determination of the most efficient model for fermentation among the two was necessary. Akaike Information Criteria (AIC) [48] is adopted for the evaluation index. Calculations for the AIC value and the Akaike weight value were made by Eq. (3) and Eq. (4) [49] for each model.

$$B = B_p \times \exp \left( - \exp \left( \frac{MBPR}{B_p} \left( BPDT - t \right) \right) \right), \quad (1)$$

$$B = \frac{B_p}{1 + \exp \left( \frac{BPDT - t}{MBPR} \right)^2}, \quad (2)$$

where:
- $B =$ volume of cumulative biogas at digestion time ($t$) (mL);
- $B_p =$ potential of biogas production (mL);
- $MBPR =$ maximum rate of biogas production (mL/h);
- $BPDT =$ biogas production delay time (h);
- $t =$ total digestion time (h).

$$\text{AIC} = \begin{cases} N \ln \frac{\text{RSS} + 2K}{N} & \text{when } \frac{N}{K} \geq 40 \\ N \ln \frac{\text{RSS} + 2K}{N} & \text{when } \frac{N}{K} < 40 \\ \end{cases}, \quad (3)$$

$$\text{Akaike's weight} = \frac{e^{-0.5 \Delta \text{AIC}}}{1 + e^{-0.5 \Delta \text{AIC}}}. \quad (4)$$

where:
- $N =$ number of points;
- $\text{RSS} =$ residual sum of square;
- $K =$ number of model parameters;
- $\Delta \text{AIC} =$ the relative difference between the two AIC values.

### 3 Results and discussion

#### 3.1 Optimization of Co NPs concentrations

The design of experiments (DOE), as suggested by the central composite design, are depicted in Table 2. As a result of the study, the estimation curve called response surface curve was obtained, as shown in Fig. 2. The color code in Fig. 2 describes the range of biogas production, with red as the highest production. The curvature in Fig. 2 depicts how the selected parameters change the biogas production. For instance, in Fig. 2(b), the most favorable and optimum design point is at 1mg/L concentration of Co NPs, while a temperature of 35 °C and pH of 7 assist the enhancement in the biogas production.

| Table 1 Independent variables selected for RSM of Co NPs |
|-----------------|-----------------|-----------------|
| Independent variables | Parameters name | Low level | High level |
| A                | Temperature (°C) | 25        | 45        |
| B                | Initial pH      | 5         | 9         |
| C                | Co NPs concentration (mg/L) | 0.5 | 2 |
On the basis of the experimental studies, the following regression model is suggested that best fits that data.

Cumulative Biogas Production = 282.992 – 1.25A
-7.87B – 3.71C + 5.74AB + 5.08AC - 5.30BC
-25.93A^2 -14.69B^2 - 15.44C^2

The validity of the mathematical regression model was studied by the ANOVA (Analysis of Variance) test, see Table 3. From the results of the ANOVA test, it is revealed that the value of R^2 (Coefficient of determination) is 0.8294, which is near to 1.00 and validates the mathematical regression model. The value of P-value (Prob>F) for Co NPs is 0.0143 that implies that there is a strong correlation between the input parameters and the biogas production. Therefore, it can be inferred that studying the impact of mentioned input parameters on biogas production is significant.

The value of A^2 Prob>F value of temperature is 0.0188 (less than 0.05) which means in the process of biogas production using Co NPs, temperature plays a significant role. In other contexts, it implies that A^2 has a strong impact on biogas production. For the case of C^2, a conspicuous impact is observed.

### 3.2 Influence on biogas production

The effect of different concentrations of the Co NPs is depicted in Fig. 3. The observations show that the addition of the Co NPs has a positive effect on biogas production. The fact that all the samples with Co NPs have a high position in the graph as compared to the control validates the enhancement in biogas production. In the case of green algae, two cell layers are present named as external and internal layers [50, 51]. The external layer is a polymer matrix consisting of carbohydrates and proteins, whereas the internal layer is made up of cellulose [23, 52–54]. Co NPs first solubilize the external layer, followed by the dissolution of the internal layer that increases the overall lysis rate, ultimately increasing biogas production. Our results are in agreement with the ones produced by Abdelsalam et al. [32]. The authors have studied the effects of various concentrations (0.5, 1, and 2 mg/L) of Co NPs on the production capability of methane and biogas from the conversion of cattle manure (CM) [32]. AD of CM was carried out batch-wise at operating temperature and mixing rate of 37 ± 0.3 °C and 90 rpm, respectively, for hydraulic retention time of 50 days. The study indicated that the addition of 1 mg/L Co NPs increases the biogas and methane volume by 1.64 and 1.86 times, respectively, for hydraulic retention time of 50 days. The study indicated that the addition of 1 mg/L Co NPs increases the biogas and methane volume by 1.64 and 1.86 times, respectively. The authors mentioned that the addition of Ni and Co NPs improve the startup of biogas production and hence, reduce the lag phase in comparison with control. Co NPs showed increased decomposition of organic matter as more decomposition of Total Solids (TS), and Volatile Solids (VS) observed at the end of the experiment.

There exists only minor difference between optimized and non-toxic Co concentration as indicated by Fermoso et al. [55]. According to Zandvoort et al. [56], the optimum dosage of Co metal is 0.8 mg/L while the dose of only 0.2

### Table 2 Design of experiments and their corresponding experimental results

<table>
<thead>
<tr>
<th>Run</th>
<th>Temperature (°C)</th>
<th>Initial pH</th>
<th>Co NPs concentration (mg/L)</th>
<th>Cumulative biogas production (mL)</th>
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</table>
mg/L Co still find significantly toxic. Our results are in agreement with this observation as 1 mg/L Co NPs concentration provided highest biogas yield. This statement is also inline with the study conducted by Qiang et al. [26] who reported that 1 mg/L Co metal dose to the digester working at thermophilic conditions resulted in good performance of biodigester. As the concentration of Co NPs increased (2 mg/L), the biogas production decreases, this observation is consistent with the results obtained by Facchin et al. [57] which indicated that inoculation of excessive Co metal concentration reduce methanogenesis process and directly affect metabolic pathway of AD process.

3.3 Mathematical kinetic models

Fig. 4 and Fig. 5 shows the values for cumulative gas production with different groups obtained from experiment as contoured plots and response surfaces; (a) Effect of initial pH and Co NPs concentration 3D plot; (b) 2D plot; (c) Effect of temperature and initial pH 3D plot; (d) 2D plot; (e) Effect of Co NPs concentration and temperature 3D plot; (f) 2D plot.
well as the prediction. Parameters of reaction kinetics calculated using the Modified Gompertz and Logistic function model are shown in Tables 4 and 5 respectively. The maximum gas production (MBPR) of the modified Gompertz model for Control, Co NPs (2 mg/L), Co NPs (1.5 mg/L), Co NPs (1 mg/L) and Co NPs (0.5 mg/L) are 5.35, 5.53, 4.72, 6.03 and 6.38 mL/hr, respectively. The MBPR for Control, Co NPs (2 mg/L), Co NPs (1.5 mg/L), Co NPs (1 mg/L) and Co NPs (0.5 mg/L) of the logistic function model were 5.53, 5.75, 4.99, 6.56, and 6.78 mL/hr, respectively. It can be found that in comparison with the other groups, Co NPs (1 mg/L) and Co NPs (0.5 mg/L) showed a better biogas production rate. The correlation coefficients of the modified Gompertz model and Logistic function model were above 99.04% and 98.67%, respectively.

Akaike Information Criteria (AIC) test results are listed in Table 6. It can be seen that the difference in yield between measured and predicted models is lower for the Modified Gompertz model as compared to the Logistic function model. AIC test confirmed that the modified Gompertz model has a lower AIC value and is considered a better choice.

4 Conclusions
The effect of Co NPs on AD of *Enteromorpha* algae was carried out in this study. The optimization of AD parameters was done by using RSM. The detailed experiment at optimized values showed that optimized concentration showed enhancement in biogas yield. Co NPs (1 mg/L) achieved 678 mL biogas. The reaction kinetics was performed by using mathematical kinetic models. Both models fitted well with the experimental data. AIC test showed that the modified Gompertz model is most suitable to use in this
case. This study lays a foundation for an improved biogas yield by Co NPs inoculation in AD of green algae. Further research is needed to explore reaction mechanisms by measuring various soluble indexes and analysis of transitional change in microbial communities due to the presence of Co NPs, which resulted in enhanced biogas production.

In this paper, the effect of only three parameters was studied on biogas production using RSM. Effect of other parameters such as retention time, working pressure, size of NPs and many more can be explored in future studies. Moreover, this study doesn't shed light on disposing off used NPs, which is creating an environmental concern these day. Studying methods to dispose off Co NPs after treatment could be a future research direction.

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<table>
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Remarks: BP: Biogas production potential; MBPR: Maximum biogas production rate; BPDT: Biogas production delay time; $R^2$: Correlation coefficient

<table>
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Remarks: BP: Biogas production potential; MBPR: Maximum biogas production rate; BPDT: Biogas production delay time; $R^2$: Correlation coefficient

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