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# Statistical Modeling of the Methane Production from Slaughterhouse Wastes in Anaerobic Co-digestion

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***Authors' contributions***

*This work was carried out in collaboration between the authors. Author YGP designed and completed the laboratory studies with support from authors YRP and MPC. Authors MHR and SRP supervised the study, supported method development and helped author YGP write up the outputs. All authors read and approved the final manuscript.*

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## ABSTRACT

A simplex-lattice mixture design and the surface response methodology (SRM) were used to modeling the methane production on the anaerobic co-digestion (AcoD) of three different substrates generated from slaughterhouses: manures (Ms), solid organic wastes (SOW) and wastewaters (SHWW). In the first stage of the study, a characterization of these residuals was carried out; meanwhile, the mixture design was used in the second step to determine the methane production obtained on the AcoD of the substrates considered. The results of the analysis of RSM show that the best adjusted model was the special cubic, with high values of $R^2$ and $R^2_{adj}$ of 95.13% and 90.96%, respectively. According to the statistical – mathematical model obtained in this study, wastes generated from slaughterhouses are appropriated material for acquire biogas; nonetheless, significant antagonistic effects was observed when increasing the amounts of SOW and SHWW, apparently by the increase in the levels of proteins and fat, oil and grease (FOGs). A good strategy to implement in order to achieve high methane productions for the effluent treatments from meat producing industries is a combination of substrates Ms and SOW; meanwhile, is preferable to separately treat the SHWW in high rate AD systems or anaerobic lagoons.

*Keywords: Statistical modeling; methane production; slaughterhouse wastes; anaerobic digestion.*

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## 1. INTRODUCTION

Several studies have showed that is feasible the anaerobic digestion (AD) process to treat the wastes generated from slaughterhouses [1-3]. The main types of slaughterhouse wastes (SHW) include the solid organic wastes (SOW) generated from meat producing industries, and the wastewaters (SHWW) are originated from the several stages of washed in the process. Those effluents show a high pollution due to the accumulation of several compounds, including blood, fat, proteins and manures (Ms). For those characteristics, the SHW are and attractive substrate for the biogas production; but, the AD process is sensitive and prone to failure due to potential inhibitory compounds can be formed during the degradation of the proteins and lipids.

AD is a biological process in which the degradation of the organic matter is accomplished by a group of microorganisms in absence of molecular oxygen, producing a gas (biogas), which is compound mainly of methane ($CH_4$), carbon dioxide ($CO_2$) and hydrogen ($H_2$). This process offers many advantages in comparison with the aerobic conventional systems, but the long times of start-up process have had a negative impact in the application to full-scale [4]. However, recent advances in the knowledge of the microbiological and biochemical processes, together with the advances of new configurations of anaerobic reactors, have promoted the interest on use those technologies for the treatment of industrial and municipal wastewaters [5,6]. The microbial populations that carry out the AD process are varied, and they establish a complex ecosystem. A single description of the process includes four main phases. In the first phase (hydrolysis), the organic complex polymers (polysaccharides, proteins and lipids) are hydrolyzed to simple and soluble organic compounds (sugars, amino acids and fatty acids). Acidogenic and acetogenic are the second and third phases, respectively, where the acidogenic microorganism degrades the intermediary groups, forming the volatile fatty acids (VFA). These VFA are subsequently turned to acetate ($CH_3COOH$) by the acetogenic bacteria, besides carbon dioxide ($CO_2$) and hydrogen ($H_2$). Finally, in the fourth stage (methanogenesis), methane is produced by methanogenic population (acetoclastic and hydrogenotrophic bacteria), mainly by the conversion of the acetate and the route of $H_2$-$CO_2$, respectively [7].

The theoretical methane yield depends on the content of carbohydrates, proteins, and fats. Due to their high lipid and protein content, these types of waste fractions have a high methane potential; the presence of lipids offers the highest yields, but with lower kinetic rates as a consequence of the slow biodegradation of lipids. Furthermore, the high content of lipids and proteins causes process instability, leading to several microbiological and operational problems when the slaughterhouse waste is treated in mono-digestion [8]. Laboratories studies are shown relatively low organic loading rates (OLRs) between 0.8 – 1.7 kg VS $m^{-3}$ $d^{-1}$ with hydraulic retention times (HRTs) of 25 -100 d have been noticed to be feasible in AD of slaughterhouse wastes [9]. AD of pig slaughterhouse waste has been studied in a few previous reactor experiments as well as in batch experiments. In batch experiments methane productions of 430 L $CH_4$ $kg^{-1}$ $VS^{-1}_{added}$ and 580 L $CH_4$ $kg^{-1}$ $VS^{-1}_{added}$ have been reported at 35ºC [10,11].

Anaerobic co-digestion (AcoD) of various organic wastes for energy production has aroused renewed interest recently. AcoD offers some important advantages: dilution of potential toxic compounds; improved balance of nutrients; synergistic effect of microorganisms; increased load of biodegradable organic matter and better biogas yields [12-14]. In spite of those advantages, it is not well investigated the impact on the methane production when are co-digested wastes from slaughterhouses factories. AcoD is a suitable option to improve the performance of the anaerobic treatment of the agro-industrial wastes. Although, several studies about the use of the AD to treat the slaughterhouse wastes and the biogas production has been carried out [15]; however, investigations about the modeling the methane production of the AcoD should to deepen. Modeling and optimization using the designs of experiments and the SRM can be useful in the adequate selection and modeling of the parameters that influence the AcoD process of the slaughterhouse wastes.

Design of experiments (DOE) is usually used in the optimization of process, when several conventional methods are compared. Inside these techniques, the surface response methodology (SRM) have the advantages of modeling mathematically and optimizing the performance of the process, minimize the variability of measure properties (denominated responses), and the decrease of operation times and global costs of production [16]. Between the diverse methodologies of SRM, the mixtures design is de special interest when the proportion of the components that constitute the mixture are considered important; also, are useful in the design and development of industrial productivity activities that can be related with formulations or mixtures. In these design, the volume remains constant (100%), due to the response will depend only of the relative proportion of the components (ingredients) in the mixture, and not of the mixture amount [17]. The interactions of two or more components, in one or several responses of interest, can be identified, modeled and optimized on the design by means of the mixture approach [18]. Based on this background, the aim of this paper was modeling statistically the methane production obtained on the AcoD generated from slaughterhouse wastes. Batch AD tests, for this purpose, were performed in order to check the methane production under different mixing ratios. Moreover, the synergic and antagonistic effects of composition of the mixture on the response variable were studied using statistical methods. Besides, a mathematical model of methane production was achieved for the control and operation of an operating anaerobic reactor treating meat producing industries.

## 2. MATERIALS AND METHODS

### 2.1 Substrates

The substrates used in the study of the AcoD were slaughterhouse wastewater (SHWW), a mixture of pig and cow manures (Ms), and solid organic wastes (SOW) generated in a meat processing industry. The SOW was a mixture of rumen and entrails rest; meanwhile, the SHWW was the liquid residual from the same process. The mixture of Ms was obtained of a storage tank for the disposal of manures located in the stockyards. The SOW was acquired on another storage tank for the store of the residuals from the slaughter. As inoculum for the batch reactors was used an anaerobic sludge from a bio-digester that treat pig manure located in a near farm.

### 2.2 Experimental Procedure

For the experiments of AD were used serological crystal bottles of 500 mL as batch reactors. All the combinations were worked in duplicated, and the average value was used for the analysis of results. In each batch reactor were dumping 50 mL of inoculum, and the amounts required of substrates. Total volume of the substrate in each reactor was maintained at 250 mL, which were homogenized through agitation before finish the inoculation stage. The evaluation period of the experiments has an around time of 32 days.

### 2.3 Mixture Design

Mixture design are used for study the effects of the components in a mixture on a response variable; where q represents the number of ingredients, and $x_i$ denotes the proportions of those ingredients (Ec. 1).

$$\sum_{j=1}^{q} x_j = x_1 + x_2 + \cdots + x_q = 1; \qquad x_j > 0; j = 1, 2, 3, \cdots, q \tag{1}$$

To maximize the methane production was use an augmented simplex-lattice design. The three substrates used as main factors were manure, solid wastes, and wastewater. The combination and proportions of those factors were optimizing using a mixture design approach. When a mixture is composing by three components, a triangle is formed, where the vertices of the triangle represent a pure component. An organized disposition consists in a uniform spaced arrangement of points in an array called simplex-lattice design. In general, a simplex-lattice design consists in m + 1, with spaced equally values among 0 to 1. This design is defined by the conditions of coordinates for obtaining the adequate data to a polynomial model of m degree with q ingredients (the proportions adopted by each component have the x-spaced values m + 1 from 0 to 1, where each variable assume the values $x_i$ = 0, 1/m, 2/m, …, m/m).

In this design, the proportions of the different substrates that compose a mixture have a value of 1 (100%). The total size of the design was 14 trials; where three trials correspond with a pure mixture (one for each component: 1, 0, 0; 0, 1, 0; 0, 0, 1), six trials with combinations of two substrates (2/3 : 1/3 : 0; 2/3 : 0 : 1/3; 1/3 : 2/3 : 0; 1/3 : 0 : 2/3; 0 : 2/3 : 1/3; 0 : 1/3 : 2/3), others three trials that correspond to the combinations of three substrates (2/3 : 1/6 : 1/6; 1/6 : 2/3 : 1/6; 1/6 : 1/6 : 2/3), and two trials on the center of the triangle (where similar proportions of all substrates are combined: 1/3, 1/3, 1/3). The assayed mixtures are shown in Table 1.

**Table 1. Mixture assayed at each point of the simplex-lattice design**

| DOE order mixture | Ms | | SOW | | SHWW | |
|---|---|---|---|---|---|---|
| | Coded value | Real value (mL) | Coded value | Real value (mL) | Coded value | Real value (mL) |
| 1 | 1 | 250 | 0 | 0 | 0 | 0 |
| 2 | 2/3 | 166.7 | 1/3 | 83.33 | 0 | 0 |
| 3 | 2/3 | 166.7 | 0 | 0 | 1/3 | 83.33 |
| 4 | 1/3 | 83.33 | 2/3 | 166.7 | 0 | 0 |
| 5 | 1/3 | 83.33 | 1/3 | 83.33 | 1/3 | 83.33 |
| 6 | 1/3 | 83.33 | 0 | 0 | 2/3 | 166.7 |
| 7 | 0 | 0 | 1 | 250 | 0 | 0 |
| 8 | 0 | 0 | 2/3 | 166.7 | 1/3 | 83.33 |
| 9 | 0 | 0 | 1/3 | 83.33 | 2/3 | 166.7 |
| 10 | 0 | 0 | 0 | 0 | 1 | 250 |
| 11 | 1/3 | 83.33 | 1/3 | 83.33 | 1/3 | 83.33 |
| 12 | 2/3 | 166.7 | 1/6 | 41.67 | 1/6 | 41.67 |
| 13 | 1/6 | 41.67 | 2/3 | 166.7 | 1/6 | 41.67 |
| 14 | 1/6 | 41.67 | 1/6 | 41.67 | 2/3 | 166.7 |

The fundamental purpose of this design is modeling mathematically the surface response, and this way predicts the response of some component or combinations of the ingredients of a mixture. Mathematical standard forms of the simplex-lattice designs are the following:

$$\text{Lineal } \left(Y = \sum_{i=1}^{q} \beta_i x_i\right) \tag{2}$$

$$\text{Quadratic } \left(Y = \sum_{i=1}^{q} \beta_i x_i + \sum\sum_{i<j}^{q} \beta_{ij} x_i x_j\right) \tag{3}$$

$$\text{Cubic } \left(Y = \sum_{i=1}^{q} \beta_i x_i + \sum\sum_{i<j}^{q} \beta_{ij} x_i x_j + \sum\sum_{i<j}^{q} \beta_{ij} x_i x_j (x_i - x_j) + \sum\sum_{i<j<k}^{q} \beta_{ijk} x_i x_j x_k\right) \tag{4}$$

$$\text{Special cubic } \left(Y = \sum_{i=1}^{q} \beta_i x_i + \sum\sum_{i<j}^{q} \beta_{ij} x_i x_j + \sum\sum_{i<j<k}^{q} \sum_{i}^{q} \beta_{ijk} x_i x_j x_k\right) \tag{5}$$

Where: Y represents the response variable of the process, $\sum_{i=1}^{q} \beta_i x_i$ represents the effects of pure components or lineal combinations, and $\sum\sum_{i<j}^{q} \beta_{ij} x_i x_j$ ; $\sum\sum_{i<j<k}^{q} \sum_{i<j}^{q} \beta_{ijk} x_i x_j x_k$ represents the effects of mixture of two and three components, respectively. When a curvature emerges in a surface response plot indicate that exist synergism or antagonism on the mixture; therefore, the use of higher-order models more elaborated (quadratic or cubic) is necessary because the investigate phenomena is complex on the experimentation region, being needed the use of a simplex-lattice design.

## 2.4 Methane Production

Statgraphics Centurion XV software was used for performing experimental design and data analysis. The efficient of AD process was investigated analyzing the response variable methane production. The response variable was daily quantified by the displacement of the liquid column in the bottle pressure. When finalizing the experimental period of 32 days, the total volume of gas produced was considered as the cumulative methane volume.

# 3. RESULTS AND DISCUSSION

## 3.1 Characterization of the Slaughter-house Wastewater

Table 2 shows a summary of the main characteristics of the SHWW used in this study. The pH observed had an average value of 8.1. In the meat producing industries, hot water is used mainly in the slaughter area; however, when it hot water is mixture with others water at ambient temperature arrive at the system treatment to values between of 24.5 – 26.6ºC. Concentrations of suspended solids are unstable, due to the fluctuation of the volume and variety of the productions. Nevertheless, the concentration of suspended solid has an average value of 15 mgSS $L^{-1}$; while, the $BOD_5$ and COD have values of 465 y 797 mg $L^{-1}$, respectively. This industry generates wastewater from the productive process and from the washing of equipment and premises, characterized by high concentrations of organic matter (COD, suspended solids and fat-oil-grease), nitrogen and phosphorus.

However, wastewater characteristics vary from plant to plant, depending on the type of industrial process and the water consumption per fowl slaughtered. The fat, oil and grease (FOG) component of high-strength wastes, such as those created in slaughterhouses, can induce several problems including clogging of pipes, adhesion to sludge causing both inhibition of mass-transfer of nutrients and sludge flotation with subsequent washout. This crust material can significantly reduce the effective volume of the digester and is largely unavailable to the anaerobic consortium as very little is accessible by hydrolytic enzymes [19].

**Table 2. Characterization of the SHWW**

| Parameters | Unit | Value |
|---|---|---|
| pH | U | 8.2±0,4 |
| Suspended solids | mg $L^{-1}$ | 15.0±13.2 |
| Conductivity | $\mu Scm^{-1}$ | 1712.7±124.6 |
| $BOD_5$ | mg $L^{-1}$ | 466±140 |
| COD | mg $L^{-1}$ | 797±87 |
| OD | mg $L^{-1}$ | 0.61±0.3 |
| Temperature | ºC | 25.4 – 26.6 |
| Total coliforms | NMP/100 mL | >1 600 |
| Fecal coliforms | NMP/100 mL | 1 133±404 |

High rate AD systems are sensitive to FOG loadings, for that reason anaerobic lagoons are currently considered the most suitable digester type for handling wastes with high FOG content. However, new research into anaerobic membrane reactor (AnMBR) technology has shown great promise in wastewater treatment, especially in wastes with high FOG loads. In spite of those advances, few investigations have involved large FOG loadings being treated using AnMBR technology. Given that high-rate AD systems are typically sensitive to FOG loadings, more research should be conducted to investigate the feasibility of FOG digestion using AnMBR technology [20]. Excessive FOG presents important challenges related to the accumulation of VFAs, which result in the acidification of the anaerobic systems. Therefore, it is significant to investigate the relationship of slaughterhouse wastes addition and digester performance, and conduct a suitable ratio of substrates to balance the digestion capacity and the methane production.

## 3.2 Methane Production

The profile of cumulated methane production of the mixtures evaluated is show in Fig. 1. Individual substrate and all combinations used were analyzed to determined el potential of the methane generated. An increment of methane production was observed in all the mixtures. Combinations 1, 3, 2 and 7 showed high methane productions, in that order respectively. All those mixtures were using high proportions of Ms, except in the last combination where only SOW was presented. Nevertheless, the highest volume of cumulated methane was observed in combination 1, where the manure was mono-digested. Generally, manures containing high ammonium concentrations which are advantageous when these are co-digested with others substrates with low N concentrations.

Usually, manure is stored at farms, producing spontaneous emissions of methane, carbon dioxide, and ammonia, thus, contributing to the greenhouse gas emissions. Animal manure is a suitable substrate for biogas production due to the presence of carbohydrates, proteins, and lipids in its composition [15]. In rural areas, liquid and solid animal manures are an important material for the AD; however, manures have commonly associated to low yields of methane. For that reason, the AcoD of manure with others organic waste materials has been used to increase the process efficiency [21,22]. The suitable co-substrates for manures are wastes C-rich, and when is possible, with high amounts of organic matter easily biodegradable. These co-substrates are characterized for elevated C-N proportions, low buffer capacity, and, depending of their biodegradability, the capacity of producing higher quantities of VFA on the AD process [23,24].

SOW generated in slaughterhouses are of a peculiar nature, particularly the ruminal content, which contains a significant amount of partially digested lignocellulosic material (grass, straw, etc.). The anaerobic conversion efficiency of lignocellulosic materials is low in bioreactors seeded with conventional anaerobic sludge, attributed to the low cellulolytic activity and specific growth rate of the anaerobic microorganisms; furthermore, the high content of lipids and proteins causes process instability, leading to several microbiological and operational problems in a anaerobic bioreactor when the slaughterhouse waste is treated [15]. During AD, protein and lipids degradation leads to the accumulation of ammonia and long chain fatty acids (LCFAs), which are well now as important inhibitors of the anaerobic microorganisms [25].

According to Pagés-Díaz et al. [26] a possible solution to solving the above-mentioned problems is the application of AcoD. In this system, slaughterhouse wastes can be treated together with the other residues generated during the agriculture activities. The main advantages of AcoD are related to a balanced nutrient supply, better C-N ratio, the dilution of inhibitory compounds, as well as to a more efficient utilization of the digester plant by treating several wastes at the same time. However, AcoD is dependent on access to available waste streams; many meat processing industries are not located within close proximity to other agro-industrial waste streams, and, subsequently, AcoD is currently not an economically viable option. For that reason, several meat processing industries which employ biogas facilities use slaughterhouse wastes as a mono-substrate [19].

On the other hand, when the proportions of the other co-substrates in the mixtures increased, the methane yields fallen. As has been shown previously, SHWW and SOW contain high FOG concentrations, which have been cited as a desirable co-digestion substrate due to its high organic contents and excellent biodegradability. Nevertheless, AcoD with excessive FOG has been regarded as an adverse condition for the application. Excessive FOG presents important challenges related to the accumulation of VFAs, which result in the acidification of systems [27]. According to Yang et al., AcoD with FOG addition presented a distinct advantage over mono-AD system due to the positive nutrition balance; however, the FOG loading in excess of 2 g $L^{-1}$ $d^{-1}$ were detrimental to biogas production [28].

### 3.3 Determination of the Mathematical Model and Statistical Evaluation

The results obtained of the mixture experimental design were analyzed using the methodology of multiple regression model, and studying as response variable the cumulative methane production (Table 3). Table 4 show the analysis of variance (ANOVA) for estimated full model effects of the response determined for four solutions: lineal (Ec. 1), quadratic (Ec. 2), cubic (Ec. 3) and special cubic (Ec. 4). For methane production, quadratic and special cubic models showed significant values (F-test calculated = $F_{cal}$ = MSS/$MSS_e$ = 5,47 and 26,0) > F-test tabulated = $F_{tab}$ = ($F_{\alpha;\ df;\ (n-df+1)}$) = $F_{0.05;\ 2;\ 9}$ = 4.26 and $F_{0.05;\ 3;\ 9}$ = 4.07), and low values of probability ($p_{quadratic}$-value = 0.0244 and $p_{special\ cubic}$-value = 0.0014) > $\alpha_{0.05}$, respectively. Fisher´s variance ratio at this level was enough to justify the suitability degree of both models.

Table 5 shows an analysis of goodness of fit to both models. Special cubic model shows the higher values of fitting ($R^2$ = 95.13; $R^2_{adj.}$ = 90.96; R = 97.53), compared with the fitting values achieve of the quadratic model ($R^2$ = 77.05; $R^2_{adj.}$ = 62.70; R = 87.78).These result indicates that for methane production the special cubic model is more significant. Special cubic model explains approximately the 95.13% of the variability of methane production. Also, a high degree of precision and a good deal of the reliability of the conduced experiments were indicated by a low values of estimation standard error (105.4 mL) and absolute mean error (60.6 mL). That results in the actual study indicate a good correlation between observes and predicted values by the special cubic model (Fig. 2). Moreover, the point cluster around the diagonal line indicates a good fit of the special cubic model.

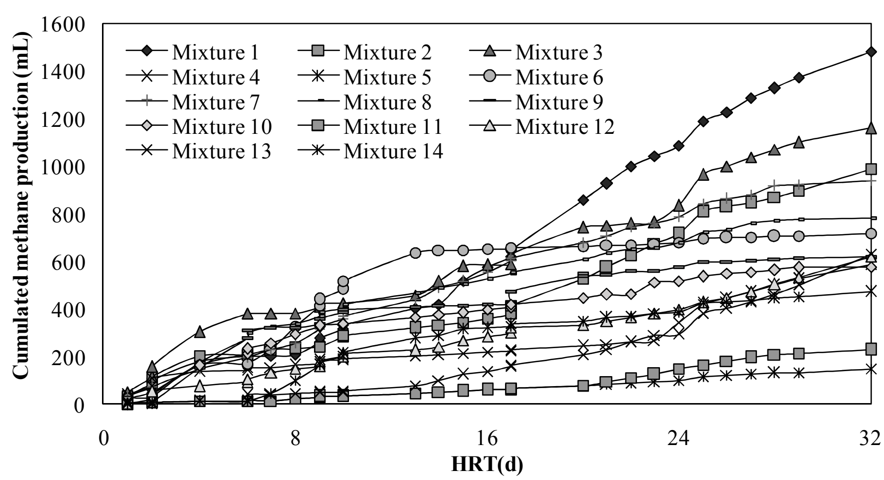


**Fig. 1. Cumulated methane production during the AcoD of mixtures from slaughterhouse wastes obtained of simplex-lattice mixture design**

**Table 3. Experimental matrix and cumulative methane production obtained of the mixtures from slaughterhouse wastes**

| DOE order | Ms | | SOW | | SHWW | | Methane production observed (mL) | Methane production predicted (mL) | Residuals | Studentized residuals |
|---|---|---|---|---|---|---|---|---|---|---|
| | Coded value | Real value (mL) | Coded value | Real value (mL) | Coded value | Real value (mL) | | | | |
| 1 | 1 | 250 | 0 | 0 | 0 | 0 | 1482.4 | 1501.0 | -18.46 | -0.44 |
| 2 | 2/3 | 166.7 | 1/3 | 83.33 | 0 | 0 | 988.1 | 880.8 | 107.40 | 1.63 |
| 3 | 2/3 | 166.7 | 0 | 0 | 1/3 | 83.33 | 1158.4 | 1080.0 | 78.01 | 1.08 |
| 4 | 1/3 | 83.33 | 2/3 | 166.7 | 0 | 0 | 584.6 | 696.4 | -111.80 | -1.73 |
| 5 | 1/3 | 83.33 | 1/3 | 83.33 | 1/3 | 83.33 | 147.3 | 214.2 | -66.93 | -0.78 |
| 6 | 1/3 | 83.33 | 0 | 0 | 2/3 | 166.7 | 718.6 | 766.7 | -48.14 | -0.63 |
| 7 | 0 | 0 | 1 | 250 | 0 | 0 | 938.7 | 948.1 | -9.384 | -0.22 |
| 8 | 0 | 0 | 2/3 | 166.7 | 1/3 | 83.33 | 782.6 | 799.5 | -16.88 | -0.21 |
| 9 | 0 | 0 | 1/3 | 83.33 | 2/3 | 166.7 | 621.3 | 670.2 | -48.91 | -0.64 |
| 10 | 0 | 0 | 0 | 0 | 1 | 250 | 574.7 | 560.3 | 14.39 | 0.34 |
| 11 | 1/3 | 83.33 | 1/3 | 83.33 | 1/3 | 83.33 | 227.3 | 214.2 | 13.07 | 0.14 |
| 12 | 2/3 | 166.7 | 1/6 | 41.67 | 1/6 | 41.67 | 620.6 | 724.2 | -103.60 | -1.11 |
| 13 | 1/6 | 41.67 | 2/3 | 166.7 | 1/6 | 41.67 | 632.1 | 480.7 | 151.40 | 1.86 |
| 14 | 1/6 | 41.67 | 1/6 | 41.67 | 2/3 | 166.7 | 470.0 | 410.1 | 59.90 | 0.60 |

**Table 4. Analysis of variance for the mathematical models evaluated for methane production**

| Source | Sum of squares | Degree of freedom | MSS[c] MSS=SS/df; $MSS_e=SS_e/df_e$ | F-value calculated[d] Fcal.=$MSS/MSS_e$ | *p*-value |
|---|---|---|---|---|---|
| Mean | $7.07 \cdot 10^6$ | 1 | $7.07 \cdot 10^6$ | | |
| Lineal model | $4.78 \cdot 10^5$ | 2 | $2.39 \cdot 10^5$ | 2.35 | 0.1412 |
| Quadratic model | $7.51 \cdot 10^5$ | 3 | $2.50 \cdot 10^5$ | 5.47 | 0.0244[e] |
| Special cubic model | $2.89 \cdot 10^5$ | 1 | $2.89 \cdot 10^5$ | 26.00 | 0.0014[e] |
| Cubic model | $4.6 \cdot 10^3$ | 3 | $1.5 \cdot 10^3$ | 0.08 | 0.9651 |
| Error | $7.31 \cdot 10^4$ | 4 | $1.83 \cdot 10^4$ | | |
| Total | $8.66 \cdot 10^6$ | 14 | | | |

[e] *p-values<0.05 were considered to be significant*

**Table 5. Full model results**

| Models | Standard error | $R^2$ (%) | $R^2_{adj.}$ (%) | R (%) |
|---|---|---|---|---|
| Lineal | 318.8 | 29.95 | 17.21 | 54.73 |
| Quadratic | 214.0 | 77.05 | 62.70 | 87.78 |
| Special cubic model* | 105.4 | 95.13 | 90.96 | 97.53 |
| Cubic | 135.2 | 95.42 | 85.12 | 97.68 |

*$R^2$ Coefficient of multiple determination*
*$R^2_{adj}$ Adjusted R-squared statistic.*
*R Correlation coefficient.*
** was considered as best model*

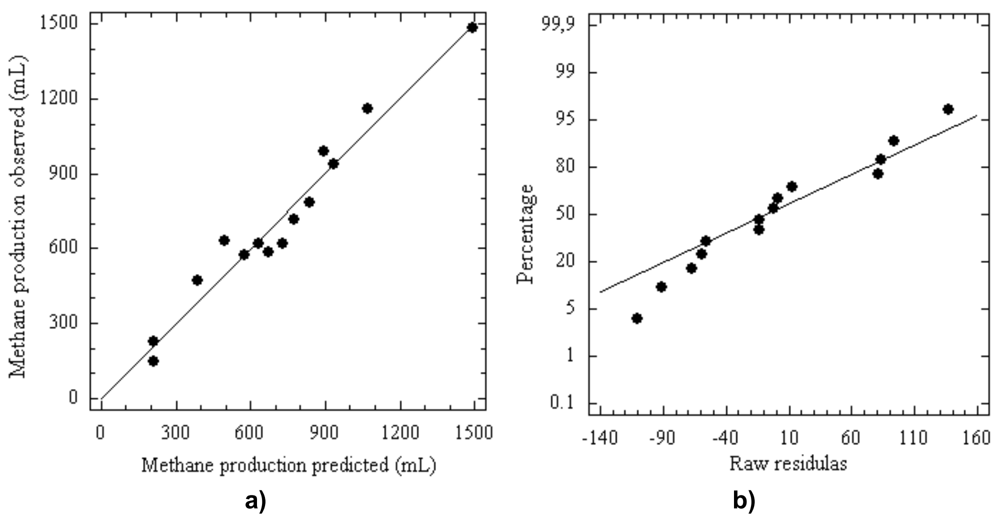


**Fig. 2. Check of the adequacy of the model: a) parity plot showing the correlation between experimental and predicted values; b) normal probability of the raw residuals**

The significance of each component of the quadratic model (Ec. 3) was determined by Student´s test, and the values are showed in Table 6. Higher magnitudes of t-statistic values and low p-values indicate the respective significant coefficient. The p-values underneath $\alpha = 0.05$ specify that the coefficients of the model are significant at 95% of probability. For methane production, all the coefficients that characterize the lineal (substrates only) and interactions between two substrates (Ms and SOW) and three terms (all substrates) are significant in the model.

On the other hand, empiric relations between methane production and the substrates selected were obtained by the application of special cubic model, where the interaction Ms*SHW and SOW*SHW was excluded because it was not being significant. The model was defined for:

$$\text{Methane production} = 1501\,\text{Ms} + 948.1\,\text{SOW} + 560.3\,\text{SHWW} - 1962\,\text{Ms} * \text{SOW} - 1.37 \cdot 10^4 \text{Ms} * \text{SOW} * \text{SHWW} \quad (5)$$

In the present work, it was possible to model the methane production the AcoD of wastes generated from slaughterhouses. The effects of two- and three-factor (substrate) interactions were included in the remaining part of the model, allowing the model to predict synergistic and antagonistic interactions. From the fitted model we can deduce the individual performance of the substrates. Ms had the higher coefficient, followed by SOW and SHWW, respectively. As is observed in the mathematical model achieve, wastes generated from slaughterhouses are appropriated material for acquire biogas; nonetheless, significant antagonistic effects was observed when increasing the amounts of SOW and SHWW, apparently by the increase in the levels of proteins and FOGs, which have demonstrated that their degradation increases the ammonium and LCFAs concentrations and they are important inhibitors of the anaerobic microorganisms [25]. Despite the fact that FOG has the potential to significantly enhance biogas yield from AD systems, FOG can also produce several problems, i.e., clogging of pipes, adhesion to sludge causing both inhibition of mass-transfer of nutrients and sludge flotation with subsequent washout [19].

On the other hand, a mixture of SOW or SHWW with Ms, where the manure is the substrate with greater proportions, considerable methane productions are obtained. Similar results were obtained by Jhosané et al., where evaluated the AcoD of solid cattle slaughterhouse waste with Ms (50% : 50%) allowed higher methane yields, with a methane content of 75% [8]. According to the authors, the addition of the manure allowed the alkalinity in the system to increase, turning to a higher buffer capacity. Hence, the system could handle the accumulation of the VFAs better.

### 3.4 Three-dimensional (3D) Response Surfaces and Contour Plots

The purpose of the mixtures optimization of different substrates for one or several responses of interest can be predicted using a technique of triangular response surface as tri-axial diagrams [18]. These graphical representations are a combination of the main factors on the representation of response. Optimal area is defined like a convex region of experimental design for which the production obtained of some proportion is higher to achieved for other proportion [29]. Through, a better understanding of the effects on the AcoD of substrates generated from slaughterhouses, a 3D plots (Fig. 3) was designated by the special cubic model (Ec. 5) to modeling the methane production. Fig. 3a and 3b show the estimate contour surface and its contours of methane production. The nonlinear nature of 3D plots demonstrated that were considerable the interactions between substrates. Higher values of methane were determinate near the vertices triangle where higher levels of Ms and low effect of SOW and SHWW were combined. These results indicate that in the AcoD of wastes generated in slaughterhouses the amount of Ms should be elevated to obtain the upper levels of methane production.

For the solution of a particular nonlinear model, each iterative step of the solver returns the best estimate found in the solution process. After each iteration, the merit function is compared with the results of the experiment. When the analysis of goodness of fit is carried out, a scientific interpretation of the obtained response is also necessary to see how well the chosen regression model truly describes the actual behavior of the experimental data. This examination should be carried out as an important task to ensure that the fitted values of any of the variables are scientifically meaningful or should not violate a possible physical reality. In some cases, depending on the characteristics of the data set, some overestimations as well as underestimations may be observed in the prediction modeling based computational studies [30].

Trace plot is a useful diagnostic tool to evaluate the importance of the components in a mixture; and that show as change the responses when each constituent is increased or decreased [17]. Fig. 4 shows trace plot of the significance of the mixture compounds, when the maximum and lowest methane productions is achieved. When increasing the proportions of Ms a favourable effect in methane production, near to its higher levels, was observed; meanwhile, an antagonistic effect was detected at raising the amounts of SOW and SHWW. From the trace plots, it was observed that the use of SOW and SHWW in major proportion was having the antagonistic effect on the response. However, the proportions of SOW can be increased to certain extent without compromising on the methane production.

**Table 6. Parameters of the special cubic model, and its significance in the methane production**

| Parameters | Coefficient | Standard error | *t*-statistic | *p*-value |
|---|---|---|---|---|
| Manure (Ms) | 1501.0 | 97.66 | 15.37 | 0.0013[e] |
| Solid organic waste (SOW) | 948.1 | 97.66 | 9.71 | 0.0033[e] |
| Slaughterhouse wastewater (SHWW) | 560.3 | 97.66 | 5.74 | 0.0094[e] |
| Ms*SOW | -1962.0 | 467.6 | -4.19 | 0.0041[e] |
| Ms*SHWW | -482.1 | 467.6 | -1.03 | 0.3369 |
| SOW*SHWW | -87.37 | 467.6 | -0.17 | 0.8571 |
| Ms*SOW*SHWW | $-1.37 \cdot 10^4$ | 2689.0 | -5.10 | 0.0014[e] |

[e] *p-values <0.05 were considered to be significant*

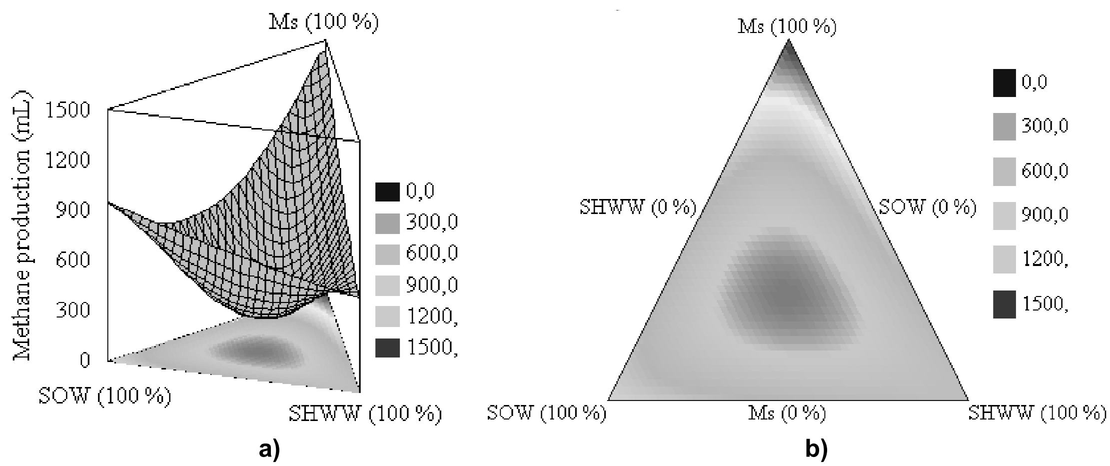


**Fig. 3. Response plots corresponding to the special cubic model for methane production: a) Estimate contour surface; b) Contours for estimate contour surface**

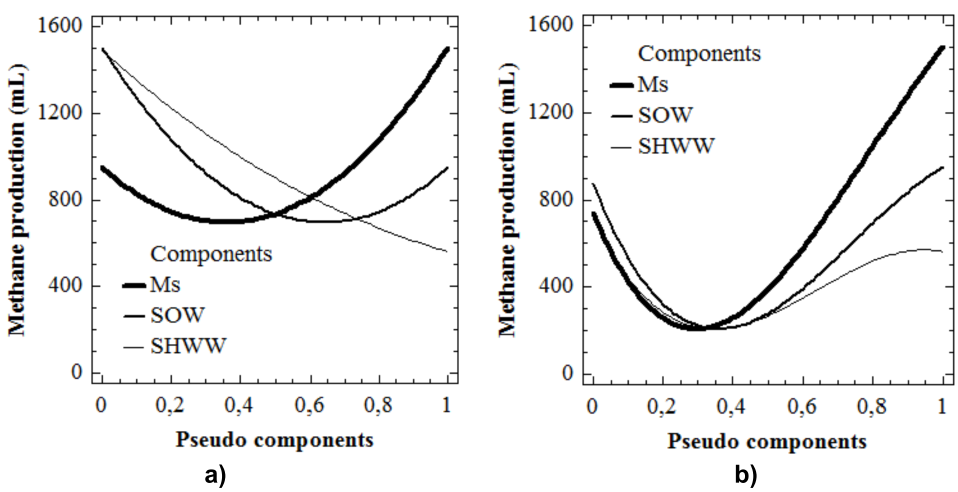


**Fig. 4. Trace plot to evaluate the significance of the mixture compounds: a) Maximum methane production; b) Lowest methane production**

In this study, the AcoD of wastes from meat producing industries was investigated by using a mixture experimental design. It was possible to relate the synergistic and antagonistic interactions obtained from the biological process to the statistical results. Little literature is available on the characteristics and quantification of organic solid by-products and wastes from slaughterhouses, though such information is needed to evaluate treatment options for these materials. A good strategy to implement in order to achieve high methane productions in large scale biogas plants, for the effluent treatments from meat producing industries, is a combination of substrates Ms and SOW; meanwhile, is preferable to separately treat the SHWW in high rate AD systems or anaerobic lagoons. However, a reliable cost/benefit analysis to better advice industry on the best course of action to provide optimal digestion of their waste, and subsequently, optimal methane production is necessary that it is considered.

## 4. CONCLUSIONS

The present paper deals about the methane production from slaughterhouse wastes and its co-digestion was investigated by using a mixture experimental design; a special cubic model has a high degree of precision to modeling the interaction between the substrates considered. From de co-digestion experiments, it was found that the mixtures containing Ms in major proportion shows the upper results, since when increasing the proportions of SOW and SHWW in the mixture, the methane yields decrease. Mono-digestion of SOW had the same effect that the co-digestion of Ms and SOW; however, a little increment in methane production was observed when Ms and SHWW were mixtures for similar proportions. RSM was used to interpret the interactions between the mixture components. Contour and 3D surface plots were more useful to find the zones where is maximized the response. This can be used in plants to generate the maximum output from the digestion process. It is important to note that it may not be always be feasible to feed a digester with optimum composition of the mixtures from slaughterhouse effluents. A good strategy to implement in order to achieve high methane productions in large scale biogas plants, for the effluent treatments from meat producing industries, is a combination of substrates Ms and SOW; meanwhile, is preferable to separately treat the SHWW in high rate AD systems (e.g. UASB reactors) or anaerobic lagoons. Furthers research is needed in order to optimize the pretreatment methods to improve the digestion of meat producing industries wastes, and its integration in the pilot- and full-scale plants, minimizing the cost and the toxicity effect of the reagent.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## REFERENCES

1. Wymyslowski M, Luczak M, Zawadzka A, Imbierowicz M, Chacuk A. Methane fermentation of poultry slaughterhouse waste. Polish Journal of Chemical Technology. 2010;12(3):15-8.
DOI: http://10.2478/v10026-010-0026-x
2. Nery VD, Pozzi E, Damianovic MHRZ, Domingues MR, Zaiat M. Granules characteristics in the vertical profile of a full-scale upflow anaerobic sludge blanket reactor treating poultry slaughterhouse wastewater. Bioresource Technology. 2008;99:2018-24.
ISSN: 0960-8524
DOI: http://10.1016/j.biortech.2007.03.019
3. Salminen EA, Rintala JA. Semi-continuous anaerobic digestion of solid poultry slaughterhouse waste: Effect of hydraulic retention time and loading. Water Research. 2002;36:3175-82.
ISSN: 0043-1354
DOI:http://dx.doi.org/10.1016/S0043-1354(02)00010-6
4. Ward AJ, Hobbs PJ, Holliman PJ, Jones DL. Optimisation of the anaerobic digestion of agricultural resources. Bioresource Technology. 2008;99:7928-40.
ISSN: 0960-8524
DOI: http://10.1016/j.biortech.2008.02.044
5. Singh S, Prerna P. Review of recent advances in anaerobic packed-bed biogas reactor. Renewable and Sustainable Energy Reviews. 2009;13:1569-75.
ISSN: 1364-0321
DOI:http://dx.doi.org/10.1016/j.rser.2008.08.006
6. Chan YJ, Chong MF, Law CL, Hassell D. A review on anaerobic-aerobic treatment of industrial and municipal wastewater. Chemical Engineering Journal. 2009;155:1-18.
ISSN: 1385-8947
DOI: http://10.1016.j.cej.2009.06.041
7. Pavlostathis S, Giraldo-Gómez E. Kinetics of anaerobic treatment: A critical review. Crit Rev Environ Control. 1991;21(5-6):411-90.
DOI:http://dx.doi.org/10.1080/10643389109388424

8. Pagés-Díaz J, Westman J, Taherzadeh MJ, Pereda-Reyes I, Horváth IS. Semi-continuous co-digestion of solid cattle slaughterhouse wastes with other waste streams: Interactions within the mixtures and methanogenic community structure. Chemical Engineering Journal. 2015;273: 28-36.
ISSN: 1385-8947
DOI:http://dx.doi.org/10.1016/j.cej.2015.03.049
9. Bayr S, Pakarinen O, Korppoo A, Liuksia S, Väisänen A, Kaparaju P, et al. Effect of additives on process stability of mesophilic anaerobic monodigestion of pig slaughterhouse waste. Bioresource Technology. 2012;120:106-13.
ISSN: 0960-8524
DOI: http://10.1016/j.biortech.2012.06.009
10. Bayr S, Rantanen M, Kaparaju P, Rintala J. Mesophilic and thermophilic anaerobic co-digestion of rendering plant and slaughterhouse wastes. Bioresource Technology. 2012;104:28-36.
ISSN: 0960-8524
DOI:http://dx.doi.org/10.1016/j.biortech.2011.09.104
11. Rodríguez-Abalde A, Fernández B, Silvestre G, Flotats X. Effects of thermal pre-treatments on solid slaughterhouse waste methane potential. Waste Management. 2011;31:1488-93.
ISSN: 0956-053X
DOI:http://dx.doi.org/10.1016/j.wasman.2011.02.014
12. Jiménez J, Guardia-Puebla Y, Cisneros-Ortiz ME, Morgan-Sagastume JM, Guerra G, Noyola A. Optimization of the specific methanogenic activity during the anaerobic co-digestion of pig manure and rice straw, using industrial clay residues as inorganic additive. Chemical Engineering Journal. 2015;259:703-14.
ISSN: 1385-8947
DOI:http://dx.doi.org/10.1016/j.cej.2014.08.031
13. Fang C, Boe K, Angelidaki I. Anaerobic co-digestion of by-products from sugar production with cow manure. Wat Res. 2011;45:3473-80.
ISSN: 0043-1354
DOI: http://10.1016/j.watres.2011.04.008
14. Alrawi R, Ahmad A, Ismail N, Kadir M-O. Anaerobic co-digestion of palm oil mill effluent with rumen fluid as a co-substrate. Desalination. 2011;269:50-7.
ISSN: 0011-9164
DOI: http://10.1016/j.desal.2010.10.041
15. Pagés-Díaz J. Biogas from slaughterhouse waste: Mixture interactions in co-digestion. In: Swedish Centre for Resource Recovery. University of Borås; 2015.
16. Montgomery D. Desing and Analysis of Experiment 8th Edition. John Wiley & Sons, Inc.; 2013.
17. Rao PV, Baral SS. Experimental design of mixture for the anaerobic co-digestion of sewage sludge. Chemical Engineering Journal. 2011;172:977-86.
ISSN: 1385-8947
DOI: http://10.1016/j.cej.2011.07.010
18. Prakasham RS, Sathish T, Brahmaiah P, Rao CS, Rao RS, Hobbs P. Biohydrogen production from renewable agri-waste blend: Optimization using mixer design. International Journal of Hydrogen Energy. 2009;34:6143-8.
ISSN: 0360-3199
DOI: http://10.1016/j.ijhydene.2009.06.016
19. Harris PW, McCabe BK. Review of pre-treatments used in anaerobic digestion and their potential application in high-fat cattle slaughterhouse wastewater. Applied Energy. 2015;155:560-75.
ISSN: 0306-2619
DOI:http://dx.doi.org/10.1016/j.apenergy.2015.06.026
20. Long J, Aziz T, de los Reyes III F, Ducoste J. Anaerobic co-digestion of fat, oil, and grease (FOG): A review of gas production and process limitations. Process Safety and Environmental Protection. 2011;90(3): 231-45.
ISSN: 0957-5820
DOI:http://dx.doi.org/10.1016/j.psep.2011.10.001
21. Bertin L, Grilli S, Spagni A, Fava F. Innovative two-stage anaerobic process for effective codigestion of cheese whey and cattle manure. Bioresource Technology. 2013;128(0):779-83.
ISSN: 0960-8524
DOI:http://dx.doi.org/10.1016/j.biortech.2012.10.118
22. Regueiro L, Carballa M, Álvarez JA, Lema JM. Enhanced methane production from pig manure anaerobic digestion using fish and biodiesel wastes as co-substrates. Bioresource Technology. 2012;123:507-13.
ISSN: 0960-8524
DOI: http://10.1016/j.biortech.2012.07.109
23. Wang X, Yang G, Feng Y, Ren G, Han X. Optimizing feeding composition and

carbon- nitrogen ratios for improved methane yield during anaerobic co-digestion of dairy, chicken manure and wheat straw. Bioresource Technology. 2012;120:78-83.
ISSN: 0960-8524
DOI: http://10.1016/j.biortech.2012.06.058

24. Astals S, Nolla-Ardèvol V, Mata-Alvarez J. Anaerobic co-digestion of pig manure and crude glycerol at mesophilic conditions: Biogas and digestate. Bioresource Technology. 2012;110:63-70.
ISSN: 0960-8524
DOI:http://dx.doi.org/10.1016/j.biortech.2012.01.080

25. Chen Y, Cheng JJ, Creamer KS. Inhibition of anaerobic digestion process: A review. Bioresource Technology. 2008;99:4044-64.
ISSN: 0960-8524
DOI: http://10.1016/j.biortech.2007.01.057

26. Pagés-Díaz J, Pereda-Reyes I, Taherzadeh MJ, Sárvári-Horváth I, Lundin M. Anaerobic co-digestion of solid slaughterhouse wastes with agro-residues: Synergistic and antagonistic interactions determined in batch digestion assays. Chemical Engineering Journal. 2014;245: 89-98.
ISSN: 1385-8947
DOI:http://dx.doi.org/10.1016/j.cej.2014.02.008

27. Kawai M, Nagao N, Tajima N, Niwa C, Matsuyama T, Toda T. The effect of the labile organic fraction in food waste and the substrate/inoculum ratio on anaerobic digestion for a reliable methane yield. Bioresource Technology. 2014;157:174-80.
ISSN: 0960-8524
DOI: http://10.1016/j.biortech.2014.01.018

28. Yang Z-H, Xu R, Zheng Y, Chen T, Zhao LJ, Li M. Characterization of extracellular polymeric substances and microbial diversity in anaerobic co-digestion reactor treated sewage sludge with fat, oil, grease. Bioresource Technology. 2016;212:164-73.
ISSN: 0960-8524
DOI:http://dx.doi.org/10.1016/j.biortech.2016.04.046

29. Rispoli F, Shah V. Optimization of the Media Ingredients for cutinase production from colleotrichum lindemuthianum using mixture design experiments. Biotechonology Progress. 2008;24:648-54.
ISSN: 1520-6033
DOI: http://10.1021/bp070280n

30. Yetilmezsoy K, Demirel S, Vanderbei RJ. Response surface modeling of Pb(II) removal from aqueous soluctiopn by *Pistacia vera* L.: Box-Behnken experimental desing. Journal of Harzadous Materials. 2009;171:551-62.
ISSN: 0304-3894
DOI: http://10.1016/j.jhazmat.2009.06.035