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Modeling of Biofoam Destabilization by Biodefoamers in Poultry Slaughterhouse Wastewater Treatment Activated Sludge

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Abstract: Biofoam formation in wastewater treatment is a challenge globally. Previously, we successfully proposed the use of biodefoamers instead of synthetic defoamers for environmental protection. In this study, we report on biodefoamation modeling using activated sludge organisms. Overall, the rate law model was determined to adequately describe foam drainage including collapse while applying biodefoamers. The target industry is the poultry processing industry whereby foam formation during wastewater treatment is an ongoing challenge.

Keywords: activated sludge; biodefoamer; biodefoamation; biofoam; foam drainage; foam collapse; synthetic defoamers; kinetic models; poultry processing; wastewater treatment

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1. Introduction

Foams in conventional activated sludge (CAS) systems are generated by forcing gas into a continuous aqueous phase that contains surface-active substances (i.e., biofoamers, suspended, and dissolved solids). Activated sludge (AS) foaming occurs due to the design and operational conditions of a wastewater treatment plant (WWTP) as well as the attributes of the wastewater [1]. Chemical foams are usually related to surfactants, whereas biological foams result from biofoamers such as *Nocardia amarae*, *Microthrix parvicella*, and their bioproducts [2]. Excessive biofoamation in WWTPs leads to poor system performance and reduces the effluent quality [3]. This leads to increased operational costs and environmental pollution. To curb the negative impacts caused by the presence of biofoam, the foam has to be destabilized.

Foams are usually unstable two-phased colloids containing polyhedral bubbles (gas) separated by a thin liquid layer called the lamella [4]. Foam destabilization occurs due to an intermittent gaseous phase, bubble coalescence, collapse, liquid drainage, lamellae breakage, and Ostwald ripening [5]. This destabilization can be enhanced by using synthetic defoamers, short-chain hydrophobic alkanes that enter the lamellae and dehydrate them [6]. Although these defoamers can be efficient in foam destabilization, they focus on removing the symptom, which is foam, and they do not prevent the proliferation of filamentous bacteria that cause foam formation. They are also relatively expensive and produce unwanted by-products such as trihalomethanes, which can damage the AS biomass. Mangundu [7] suggested that novel approaches are required to overcome the shortcomings of the existing strategies. The presence of a biodefoamer consisting of multiple charged functional groups containing both hydrophilic and hydrophobic attachment sites might

enhance the attachment of the filamentous bacteria into the AS flocs, depriving foam formers of the FOG required for their growth, meaning that the number of filaments can be reduced and the biofoam lessened [8].

Liu et al. [9] suggested using specific bacteriophages with a mono spectrum to reduce biofoamers and reported that using GordTnk2, Gmal1, GordDuk1, and Gsput1 reduced Gordonia tenfold in wastewater. Dyson et al. [10] explicitly isolated a target bacteriophage from the wastewater it was tested against. One bacteriophage managed to reduce *Skermania piniformis*, which is a foam-forming bacterium. Two polyvalent DNA bacteriophages, GTE2 and GTE7, lysed multiple spectrums of biofoamers [11]. Pajdak-Stos et al. [12] used rotifers (i.e., *Lecane tenuiseta*, *Lecane inermis*, and *Lecane pyriformis*) to reduce *M. parvicella* and Eikelboom Type 0092 filaments. However, the use of biological cells or their bio-products (biodefoamers), isolated directly from the poultry slaughterhouse wastewater (PSW) and tested for their activities in this study, have limited coverage in the literature.

Kinetic modeling of biofoam destabilization is required to understand processes such as foam drainage and foam collapse, which result in foam destability in the presence of a biodefoamer or a synthetic defoamer [6]. Hence, this research aimed to develop a kinetic model that determines foam destabilization during PSW treatment in the presence of a biodefoamer and quantifies the factors involved in foam destabilization. Furthermore, comparing existing mathematical models and their ability to predict biofoam defoamation was deemed prudent. This was undertaken by first generating the data for foaming potential and foam stability including destabilization, and second, using the data to determine the kinetic model parameters (i.e., rate constants) that were applied to reproduce the foam destabilization profile under assessment. This study highlights the process that causes foam drainage and decay in the presence of a biodefoamer. This will help to effectively regulate the activated sludge requirements and contribute to the system optimization and effective activated sludge operation that can be applied in PSW treatment.

According to our knowledge, this is the first study to report on foam destabilization kinetics when a biodefoamer is used to reduce foaming during PSW treatment (i.e., in PSW/AS systems).

Theory: Kinetic Models

In this study, several kinetic models were used to elucidate foam destabilization in the presence of a biodefoamer in comparison to a synthetic defoamer. The foam destabilization rate can be described as in Equation (1).

$$-\frac{dh}{dt} = r_a = k_i h^n \quad (1)$$

where r_a is the rate of foam destabilization rate, k_i is the kinetic defoamation constant, h is the foam height or liquid height obtained by calculating foam overrun, and n is the model fitting constant. When n is 0, it means that the rate of destabilization does not depend on the concentration of the defoamer, whereas when n increases to 1 or 2, foam destabilization will solely depend on the concentration of the defoamer.

Furthermore, Monod's kinetic model was also used (—see Equation (2)), where k_m is the maximum specific defoamation rate, h is the foam height, while k_y is half the maximum specific defoamation rate.

$$-\frac{dh}{dt} = \frac{k_m \cdot h}{k_y + h} \quad (2)$$

2. Materials and Methods

2.1. Batch Culture Experiments to Produce Biodefoamers

Isolates (n = 4) of *Bacillus subtilis* (GCA_000009045.1), *Aeromonas veronii* (GCA_000204115.1), *Klebsiella grimontii* (UGJQ01000001.1), and *Comamonas testosteroni* (GCA_900461225.1) were grown on nutrient agar plates overnight (24 h, 37 °C). These isolates were previously determined to produce defoamers individually, with higher defoamer production capabilities

when used as a consortium at an optimum pH 7 and concentration of 4% (*v*/*v*) [13]. A loop-full of each isolate was inoculated into a 50 mL nutrient broth (pH 7) in 250 mL conical flasks subsequent to incubation and swirling at 37 °C and 120 rpm (Labwit ZWYR-240 shaking incubator, Labwit Scientific, Burwood East, VIC, Australia) for 24 h to produce a consortium seed culture. Next, 96 mL of a nutrient broth media at pH 7 was inoculated with 4 mL of the seed culture and incubated at 37 °C overnight (24 h) to produce defoamers. The fermentation broth was centrifuged (Hermle-Z233M-2 centrifuge, Labortechnik GmbH, Wasserburg, Germany) to recover the biodefoamers from individual flasks in 2 mL Eppendorf tubes at 15,000 rpm for 30 min to recover a biomass-free supernatant. Supernatant biodefoamer-containing samples (from n = 3 flasks) were pooled to obtain a crude biodefoamer sample to be used in the defoamation assessments.

2.2. Set-Up for Foaming Potential (Foamability) of the Activated Sludge Samples

Five liters of AS-containing wastewater was collected using sterile 10 L polypropylene bottles from a municipal WWTP in Scottsdene, Cape Town, South Africa. The experiments were conducted using the laboratory scale aeration apparatus shown in Figure 1 (3 cm diameter and 24.5 cm height, 4.6 cm diameter, and 29.4 cm height as well as 6.2 cm diameter and 49 cm height). This was carried out to determine the foamability and stability of the AS-containing wastewater. To generate foam, the air was sparged at various air flow rates (14, 7, 3.5, 0.7 mL/min) through an air stone using an aeration pump coupled with an airflow meter, as described elsewhere [13]. The sample volumes were also varied (50, 100, and 250 mL) and aerated for various times (10, 20, 30 s). These experiments were conducted at room temperature.

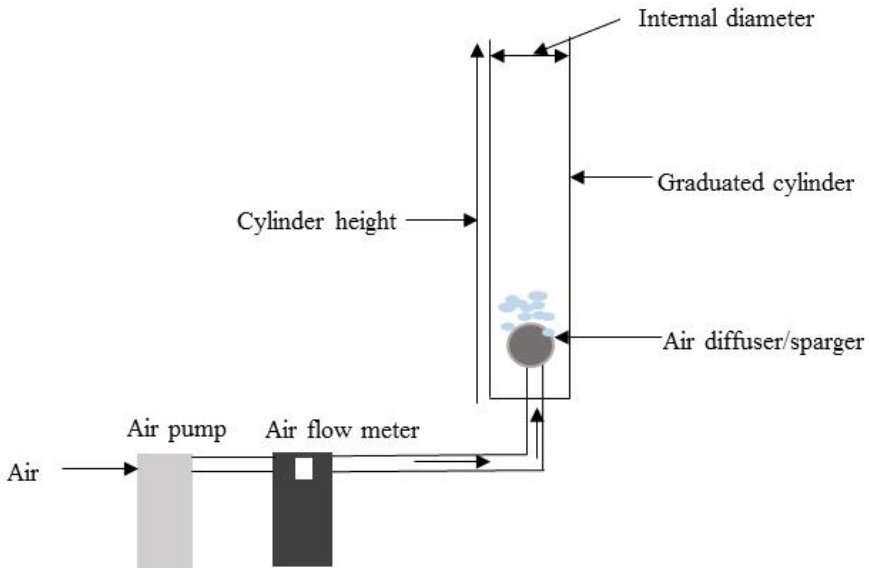

Figure 1. Foaming apparatus setup.

Equation (3) was used to calculate the foaming potential (FP).

$$FP\left(\mathrm{mL{\cdot}L^{-1}}\right) = \frac{(H_0 - H_t)\,S}{Q{\cdot}\mathrm{t}} \tag{3}$$

where H_0 is the initial liquid height before aeration (cm), H_t is the foam–liquid interface height post aeration (cm), S is the cylinder area (cm^2), Q is the air flow rate L_{air}/min, and t is the aeration time (min).

The foam height (H_t, cm) during foam collapse (i.e., foam decay) after the discontinuation of air sparging was used to determine the foam stability—see Equation (4), where k^* is the foam decay rate constant (min^{-1}).

$$H_{(t)} = H_0 \times e^{(-k^* \cdot t)} \quad (4)$$

2.3. Foam Destabilization Kinetic Parameter Assessments during Poultry Slaughterhouse Treatment

For the foam destabilization tests, a combined volume (1.8 L) of PSW and AS (filament index of 4–5), in a ratio of 1:2, was added into 2 L bioreactors (Schott bottles). Biodefoamers were as produced in Section 3.1; however, the time was elongated to 48 h. For each 2 L mixture of the collected PSW and AS, 4% v/v of the biodefoamers was added into the PSW-AS mixture, and subsequently swirled and stored at room temperature for 10 days. Sampling was carried out every 24 h. Before sampling, the mixture was swirled for 5 min, after which a volume of 200 mL was added into the (500 mL) foaming column (Figure 1). To mix the samples, air was sparged at 40 mL/min, after which foam was generated by sparging air through an air diffuser at a flow rate of 0.9 L/min until a foam volume of 50 mL was generated, and the air pump was switched off for 40 s. Additionally, foam destabilization in the form of foam collapse rate (FCR), which is directly related to foam drainage, was determined using the Ross–Miles method with minor modifications [14]—see Equation (5).

$$\text{FCR(L/min)} = \frac{\text{foam in the column after sparging}}{\text{time taken to collapse the entire foam}} \quad (5)$$

2.4. Regression and Statistical Analysis

Mathematical models that describe foam decay were fitted into the experimental data and a nonlinear regression function in Polymath v6.0 (Polymath®®, NJ, USA) was used to estimate the value of the kinetic parameters; thereafter, the simulated data containing the estimated kinetic parameters were used to compare the simulated data to the experimental data. The mean was calculated as shown in Equation (6).

$$Mean = \overline{x} = \frac{\sum x}{n} \quad (6)$$

where $\sum x$ is the sum of data points, while n is the number of experiments conducted.

Furthermore, sample variation was determined as a measure to determine the data dispersion, assessing the spread of the data using Equation (7):

$$S_y^2 = \frac{\sum (x_t - \overline{x})^2}{n-1} \quad (7)$$

where S_y^2 is the sample variance, and x_t is the value of the one observation at a specific time during experimentation. The sample variance was determined during the quantification of kinetic parameters for models selected for the foam destabilization assessments.

Similarly, the kinetic modeling standard deviation was determined as follows (Equation (8)):

$$S_x = \sqrt{\frac{\sum (x_t - \overline{x})^2}{n}} \quad (8)$$

where S_x is the standard deviation.

3. Results and Discussion

3.1. Foaming Potential (Foamability) of the Activated Sludge

Foamability is the consumed sample volume from sparging 1 L of air [15]. It is crucial to determine the sample's foamability/foam potential because it leads to foam stability [6],

and it is an intrinsic factor of the wastewater to be tested [3]. Figure 2 illustrates the foamability of the sample and the effect of AS concentration on the foaming potential. For each of the tested samples, the foamability increased as the concentration of AS increased due to planktonic extracellular polymeric substances (EPSs) [16]. This was attributed to sludge age, with foaming occurring as the sludge grew older than eight days [17].

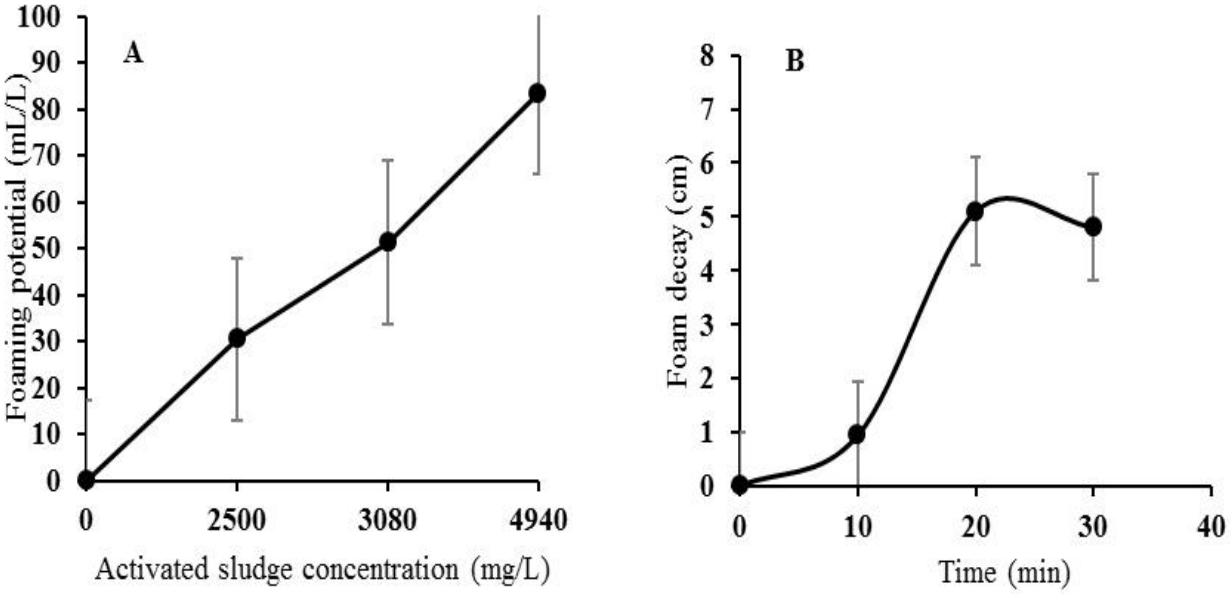

Figure 2. (**A**) The effect of activated sludge concentration on foaming potential; (**B**) biofoam decay rate.

The AS used for this study was dominated by foam formers such as *Gordonia kroppenstedtii*, *Candidatus Microthrix parvicella*, *Norcardioides insulae*, and *Bacteroides nordii*. These filamentous microorganisms are hydrophobic actinobacteria that contain mycolic acid, which helps them to attach to the gas bubbles [18]. If they reach a critical micelle concentration, they cause inter-floc deflocculation, which leads to foaming and bulking of the AS; moreover, their surface activity will increase [1,19]. The foaming of the activated sludge leads to the carrying over of suspended solids to the effluent of the primary aeration tank, leading to the WWTP system's inefficiency [20].

Foam stability was determined by using foam decay rate tests using the AS. These tests conveyed different results compared to the literature reviewed. Usually, foamability affects the foam stability; however, these two were not interrelated in this study. The highest foam stability of 5.1 cm was achieved using a 150 mL sample in a 250 mL foaming rector at a 3080 mg/L AS concentration, with a foaming potential of 51.3 mL/L. Meanwhile, the lowest foam stability of 0.94 cm was achieved using a 250 mL sample with 4090 mg/L AS concentration in a 500 mL foaming reactor. This was the highest concentration of AS used in this study, with a foaming potential of 83.5 mL/L. Therefore, reactor design specifications such as volume and diameter play a crucial role in the foam stability of the samples. Conventionally, foamability is portrayed as directly proportional to foam stability; however, this study proved that this is not always true. Foamability in this study was not affected by the reactor designs; however, foam stability was, a phenomenon attributed to air exposure at the top of the cylinder in the experiments.

3.2. Foam Drainage (Collapse) Kinetics

Foamability and stability are immensely affected by foam drainage and collapse, which results from the pressure difference within the foam as well as the pressure gradients between the plateau edges of the lamellae, which result in lamellae rupture and foam destabilization [5,21]. The rate law and Monod's model were used to evaluate and estimate the foam drainage. This was observed by simulating the estimated model data in

correlation with the experimental data using Polymath v6.0 software, which predicted the kinetic constants of each model by graphically illustrating the parity plots to determine the interrelation between the predicted and experimental values. The rate law and Monod's model better described the foam drainage experimental data, with the determination coefficients of (R^2) 1 and 0.99, respectively. Table 1 lists the computed kinetic parameters for foam drainage.

Table 1. Predicted kinetic parameters for the foam collapse rate (foam drainage).

Model	k_m	k_i	k_y	n	R^2	Variance
Rate law	-	9	-	−14.62	1	6.99×10^{-19}
Monod's	−0.988	-	0.099	-	0.99	0.015

The adjusted R^2 for the rate law and Monod's model kinetics were 1 and 0.99, respectively. The highest adjusted R^2 indicated that the rate law and Monod's models significantly predicted the kinetic constants. The lowest variance of 6.99×10^{-19} and 0.015 showed that the estimated model produced using the predicted values indicated that they were similar to the experimental data. These models gave a better fit with a standard deviation of 2.2×10^{-10} and 0.034, respectively. Ross and Miles [14] studied foam drainage kinetics using a similar model to the rate law, while Pal et al. [22] studied foam defoamation kinetics using a sonicator as a defoamer. Wang et al. [23] also studied the foam drainage kinetics, indicating that the values obtained there concur with those obtained in this study.

3.3. Foam Decay Kinetics

Foam collapse kinetics were analyzed using two models (generic rate law and Monod's). This was achieved by simulating the predicted model and comparing it to the generated experimental data, additionally plotting them together to determine the relationship between the model and the experimental data. The experimental data were fitted to the model so that kinetic constants could be predicted using Polymath v6.0 software. The generic rate law model could describe the experimental data of foam decay. The coefficient R^2 was 0.98 (Table 2).

Table 2. Estimated kinetic parameters for foam decay rate.

Model	k_m	k_i	k_y	n	R^2	Variance
Rate law	-	0.00011	-	0.781	0.98	0.0148
Monod's	5.96	-	0.439	-	0.62	0.038

The adjusted R^2 for the rate law and Monod's decay kinetics were 0.98 and 0.58, respectively. The highest adjusted R^2 indicates the significance of the model and its predicted kinetic constants. The significant model for this part of the study was the rate law model. The minute variance of 0.0148 for the rate law model depicted that the estimated model produced, using the predicted parameters, was similar to the experimental data and gave a better fit (R^2 = 0.98, adj R^2 = 0.98, variance = 0.0148, and a standard deviation = 0.001). Monod's model had a poor estimation of the foam decay parameters (R^2 = 0.62, adj R^2 = 0.58, variance = 0.038, and standard deviation of 0.0053).

Polyuzhyn et al. [24] studied the effect of a silicone defoamer on foam destabilization using an exponential decay model; in this study, the rate at which the foam decayed/collapsed was proportional to the foam height; hence, the volume and height in this equation can be used interchangeably. Polyuzhyn et al. [24] obtained an R^2 of 0.99, adj R^2 of 0.98, variance of 0.021, and a standard deviation of 0.00011. The foam collapse rate resembled a second-order degradation response [25]. In Figure 3A, the R^2 of Monod's equation was 0.77, which was higher when the data were assimilated into the experimental values. Since the model did not show consistency, it was not a better fit for the experimental

data used in this study. However, the R^2 for the generic rate law was consistent with the assimilation plot; hence, it was a better fit for foam decay prediction. The mathematical model that could predict biodefoamation was the generic rate law model.

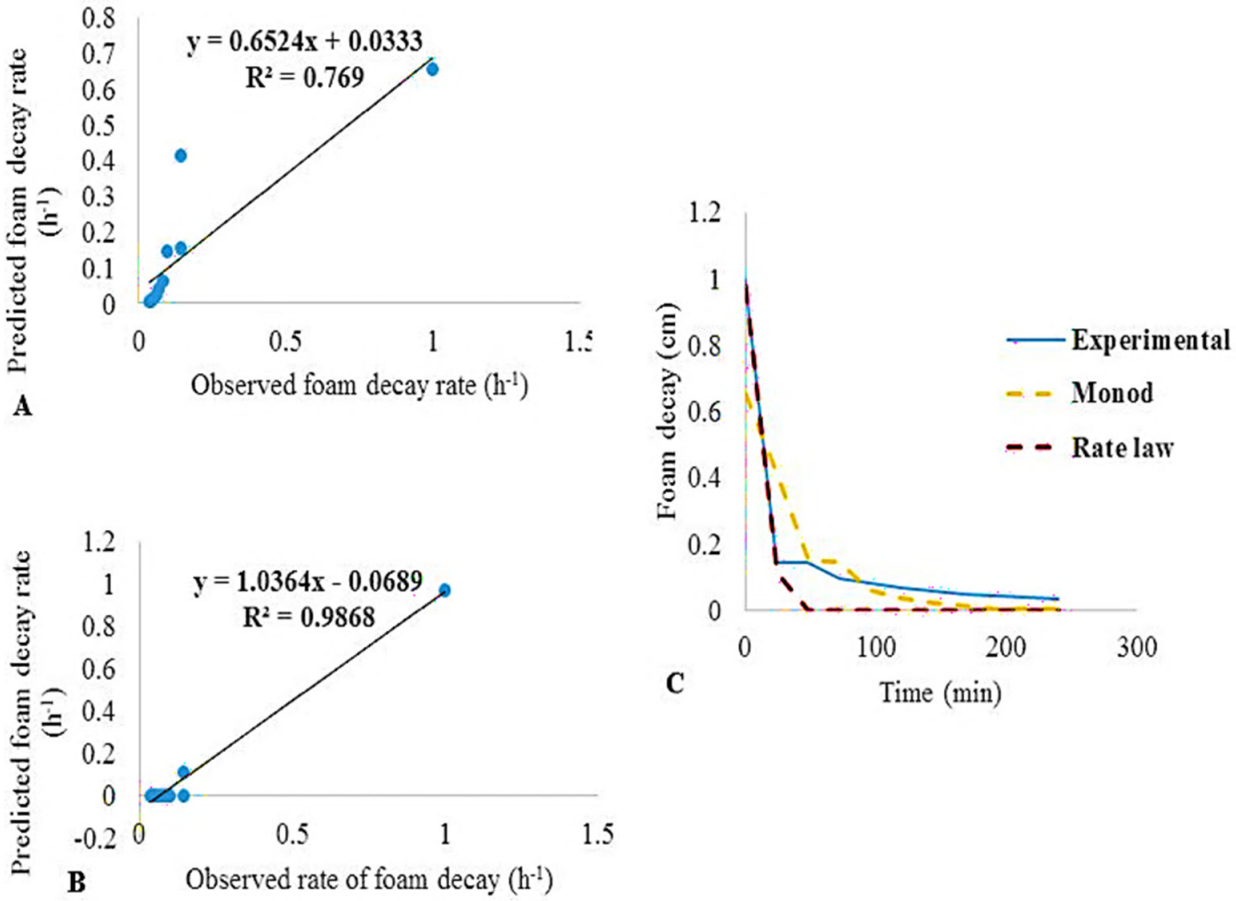

Figure 3. Consistency plots of (**A**) Monod's model, (**B**) the rate law, and (**C**) illustration of the model plots in comparison to experimental foam decay data.

For the PSW/AS system, our interest was in the foam dissipation; therefore, consistency plots for predicting foam decay were necessary. This was conducted by comparing the predicted foam rates to those observed experimentally—see Figure 3.

4. Conclusions

The highest foam stability of 5.1 cm was achieved using 250 mL and a low foaming potential of 51.3 mL/L. The lowest foam stability of 0.94 cm was achieved using 500 mL with the highest foaming potential of 83.5 mL/L. Therefore, the reactor specifications played a considerable role in both the foamability and foam stability. The rate law model is a mathematical model that could predict biodefoamation in both the foam drainage and collapse better than the use of Monod's method in this study. This model could predict foam drainage and collapse with high R^2 and adjacent R^2 values. Moreover, it had low values for variance and standard deviation, which are required for a good predictive model. Although this consortium's capability to destabilize biofoam is high, its biological and physicochemical attributes require more exploration for a better understanding of the mechanism that is used by these microbes for biodefoamation and to optimize the biofoam destabilization efficiency.

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