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Carbon dioxide is the most influential gas in greenhouse gasses and its amount in the atmosphere reached 412 µmol/mol in August 2020, which increased rapidly, by 48%, from preindustrial levels. A brand-new chemical industry, namely organic chemistry and catalysis science, must be developed with carbon dioxide (CO2) as the source of carbon. Nowadays, many techniques are available for controlling and removing carbon dioxide in different chemical processes. Since the utilization of CO2 as feedstock for a chemical commodity is of relevance today, this study will focus on how to increase CO2 solubility in culture media used for growing microbes. In this work, the CO2 solubility in a different medium was investigated. Sodium hydroxide (NaOH) and monoethanolamine (MEA) were added to the culture media (3.0 g/L dipotassium phosphate (K2HPO4), 0.2 g/L magnesium chloride (MgCl2), 0.2 g/L calcium chloride (CaCl2), and 1.0 g/L sodium chloride (NaCl)) for growing microbes in order to observe the difference in CO2 solubility. Factors of temperature and pressure were also studied. The determination of CO2 concentration in the solution was measured by gas analyzer. The result obtained from optimization revealed a maximum CO2 concentration of 19.029 mol/L in the culture media with MEA, at a pressure of 136.728 kPa, operating at 20.483 °C.

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Determination of Dissolved CO_2 Concentration in Culture Media: Evaluation of pH Value and Mathematical Data

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Abstract: Carbon dioxide is the most influential gas in greenhouse gasses and its amount in the atmosphere reached 412 μmol/mol in August 2020, which increased rapidly, by 48%, from preindustrial levels. A brand-new chemical industry, namely organic chemistry and catalysis science, must be developed with carbon dioxide (CO_2) as the source of carbon. Nowadays, many techniques are available for controlling and removing carbon dioxide in different chemical processes. Since the utilization of CO_2 as feedstock for a chemical commodity is of relevance today, this study will focus on how to increase CO_2 solubility in culture media used for growing microbes. In this work, the CO_2 solubility in a different medium was investigated. Sodium hydroxide (NaOH) and monoethanolamine (MEA) were added to the culture media (3.0 g/L dipotassium phosphate (K_2HPO_4), 0.2 g/L magnesium chloride ($MgCl_2$), 0.2 g/L calcium chloride ($CaCl_2$), and 1.0 g/L sodium chloride (NaCl)) for growing microbes in order to observe the difference in CO_2 solubility. Factors of temperature and pressure were also studied. The determination of CO_2 concentration in the solution was measured by gas analyzer. The result obtained from optimization revealed a maximum CO_2 concentration of 19.029 mol/L in the culture media with MEA, at a pressure of 136.728 kPa, operating at 20.483 °C.

Keywords: carbon dioxide; culture media; microorganism; optimization

1. Introduction

Fossil fuels are broadly acknowledged as being the principal source of energy, and since the First Industrial Revolution the amount of carbon dioxide (CO_2) in the atmosphere has risen from 280 μmol/mol to 412 μmol/mol. The resulting CO_2 emissions contribute significantly to worldwide climate change [1]. Up to now, the deployment of cutting-edge low-carbon fossil-energy technologies was considered to be the ultimate solution. Preventing worldwide climate change can be achieved by taking two long-term emission objectives into account. First, CO_2 emissions have had to reach their highest point, and then in the second half of the century, the goal has had to be to strive to achieve net greenhouse gas neutrality, by balancing anthropogenic emissions by the sources with the removal by sinks [2]. Hence, it is crucial to decrease such anthropogenic emissions. Second, CO_2 can be captured and used as a significant feedstock to produce valuable commodities. As the world population increases, the need for energy supply rises at an exponential rate. Subsequently, to meet this demand, new and renewable energy sources are required. Along this line, treating CO_2 as a feedstock to many value-added chemicals and fuels addresses both emission-control and energy supply challenges [3].

The concepts mentioned above are commonly used in carbon management from a climate change perspective. The term used is CO_2 capture, utilization, and sequestration (CCUS). The carbon capture and storage (CCS) approach in reducing CO_2 emissions is particularly common nowadays [4]. It refers to technologies that emphasize the selective removal of waste CO_2 from a large point source, its compression into a liquified gas, and finally its transportation and sequestration to a storage site where it will not enter the atmosphere such as underground geologic formations, including depleted oil and gas reservoirs or oceans [5]. Meanwhile, carbon capture and utilization (CCU) technologies capture CO_2 to be recycled for an additional application. It differs from CCS in that CCU does not permanently sequester the CO_2 waste, but rather, treats it as a renewable carbon feedstock to complement the conventional petrochemical feedstocks for conversion into other substances or products with higher economic value [6].

However, due to the thermodynamically stable nature of CO_2, utilizing it in chemical reactions is challenging. High energy input is required to breakdown carbon atoms in CO_2 molecules, which is one of the reasons why CO_2 is not extensively used in current chemical industries. Nevertheless, autotrophic microorganisms are well-known for their ability to utilize light to fix atmospheric CO_2 during the process of photosynthesis. These microorganisms can capture energy in the light cycle and store it for converting adenosine diphosphate (ADP) and nicotinamide adenine dinucleotide phosphate (NADP) into adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate hydrogen (NADPH) respectively. They then utilize these energy molecules during a dark cycle for transforming CO_2 into valuable organic compounds [7]. Research has been done recently on altering the molecules of autotrophic cyanobacteria and algae through metabolic engineering to take advantage of their abilities to treat CO_2 [8]. Microorganisms require macronutrients, micronutrients, and vitamins to grow [9]. Based on these requirements, a culture broth is required as a growth medium in a closed system, such as a bioreactor, for the production of biomass or organic compounds [10]. Therefore, increasing the CO_2 solubility in a culture broth is an important step toward CO_2 utilization by microorganisms.

Al-Anezi et al. studied the effect of temperature, salinity, and pressure on CO_2 solubility in different aqueous solutions [11]. The relationship between these parameters on CO_2 solubility was presented. Where gas solubility reduced between the temperatures of 25 °C and 60 °C, the effect was less evident at a higher temperature. Meanwhile, higher pressure (one to two bars) resulted in higher gas solubility. Additionally, the study stated that gas solubility decreased as salt increased in the solution. Yincheng et al. compared the CO_2 removal efficiency of sodium hydroxide (NaOH) and aqueous ammonia [12]. The study involved the capture of CO_2 in a spray column, and a fine spray of ammonia and NaOH was used. The key finding of the study was the value of mole ratios of NaOH and ammonia to CO_2 suitable for the spray column, which is 4.43 and 9.68 respectively. Additionally, Martins da Rosa et al. researched the CO_2 fixation of *Chlorella* using monoethanolamine (MEA) [13]. Through this research, it was found that the CO_2 intake was higher for the growth of algae using a certain mass concentration of MEA; 50 mg/L and 100 mg/L for this particular strain of algae. However, the treatment of CO_2 decreased if the MEA concentration used was very low or very high. Thus, the concentration of MEA used was dependent on the microorganism used.

The present paper will investigate various features that determine the concentration of CO_2 dissolved in a culture solution. First, to determine the maximum CO_2 concentration in the NaOH aqueous solution as a comparison, a steady rate of CO_2 was supplied for a certain time. NaOH was chosen because the CO_2 absorption capacity of NaOH solution is high, with a mass ratio of capture, $w(NaOH/CO_2)$ equal to 0.9 [14]. Second, MEA and culture media were used as the absorbent to determine the capability of both solutions in capturing CO_2. Next, different kinds of culture media solutions were prepared; with the addition of either NaOH or MEA, and the absorption was carried out under the same conditions as the previous run in a batch reactor. From the experimental results, the absorption behavior is presented according to pH and time. Then optimization was run by software to determine the optimized condition for CO_2 absorption.

2. Materials and Methods

2.1. Carbon Dioxide (CO₂) Delivery System

2.1. Carbon Dioxide (CO$_2$) Delivery System

A batch-typed glass (borosilicate) cylindrical reactor (Bio Gene®, Australia) with a built-in motor, the total volume capacity of 5 L (D = 140 mm; h = 325 mm), equipped with a pressure gauge, (RS Components, Johor Bahru, Johor, Malaysia), pH (BOQU®, Shanghai, China) and temperature probe (DPSTAR Manufacturing Sdn. Bhd., Kuala Lumpur, Malaysia) was employed for the carbon dioxide (CO_2) absorption as shown in Figure 1. The reactor was connected to a pressurized gas mixture tank (Gaslink Industrial Gases Sdn. Bhd., Puchong, Selangor, Malaysia) through a flowmeter (HERO TECH®, Puchong, Selangor, Malaysia) with a valve for controlling the flow rate of the mixture. The compositions of the gas mixture were 90% CO_2 and 10% Nitrogen (N_2). For providing a vacuum space inside the reactor, a vacuum pump (vacuubrand®, Wertheim am Main, Baden-Württemberg, Germany) was mounted with a valve. The gas analyzer (Geotech Environmental Equipment Inc., Denver, CO, USA) was connected through one of the openings to determine the amount of CO_2 in the headspace. The unwanted opening at the surface of the reactor was closed by a stopper. Additional CO_2 diffuser (FunPetAqua.my, Kuala Lumpur, Malaysia) was employed to increase the CO_2 absorption rate. All dimensions and components of the diffuser are described in Figure 2. All components were made of borosilicate except for the distributor head that was made of porous rubber.
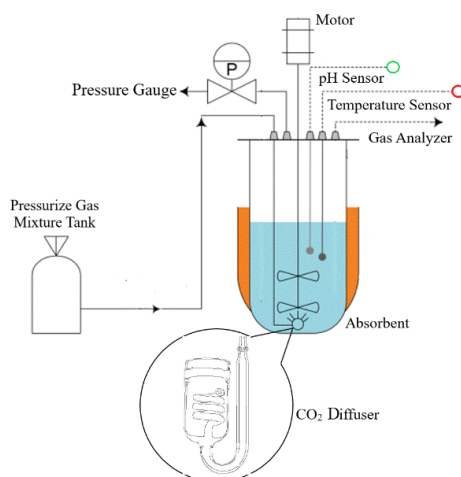

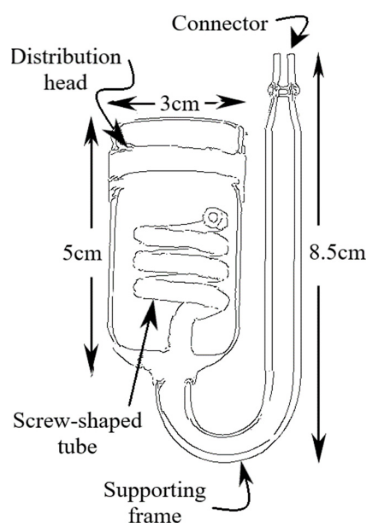**Figure 1.** Carbon dioxide absorption system.

Figure 2. Carbon dioxide diffuser.

2.2. Time Measurement of The Maximum Carbon Dioxide (CO_2) Dissolved

An aqueous solution with the concentration of sodium hydroxide (NaOH) equal to 0.1 mol/L was prepared by dissolving sodium hydroxide pellets (Sigma-Aldrich, St. Louis, MO, USA) in 2 L of distilled water. Before absorption was conducted, the absorbent (NaOH) temperature was maintained at room temperature, 100 kPa pressure and pH = 11.0 by adding hydrochloric acid (HCl) (Sigma-Aldrich, St. Louis, MO, USA) or NaOH to adjust the pH value to a desirable number. All equipment, including tubes, fittings, and the headspace of the reactor, were sufficiently washed by a vacuum pump. After the conditions are met, the absorption was carried out by injecting the gas mixture into the absorbent via a sparger with a flow rate of 4.5 L/min controlled by a mass flow controller. The solution was mixed using a mechanical stirrer at a speed of 180 rpm for uniform reaction in an absorber. The variations of pH during the reaction were measured every 10 s. The gas mixture was bubbled into the absorbent until the pH value stops dropping. The time for the CO_2 component in the mixture to dissolved in the medium was taken and a graph for pH drop against time was drawn. The same experiment was repeated while using 0.1 mol/L monoethanolamine (MEA) (Sigma-Aldrich, St. Louis, MO, USA) solution to determine the pH drop for both absorbents.

Then the experiment was repeated by replacing the absorbent with culture media. Culture media composed of 3.0 g/L dipotassium phosphate (K_2HPO_4), 0.2 g/L magnesium chloride ($MgCl_2$), 0.2 g/L calcium chloride ($CaCl_2$), and 1.0 g/L sodium chloride (NaCl) was prepared (Sigma-Aldrich, St. Louis, MO, USA). A total of 0.1 mol/L aqueous NaOH solution or 0.1 mol/L MEA solution was added to culture media to promote CO_2 absorption. It is important to determine media capability as CO_2 absorbent as culture media will help microbes to utilize CO_2 for producing the chemical commodity. The parameter is set as the previous run, until the CO_2 concentration is maximized, and then the gas mixture supply will be stopped. The graph of pH drop against time for both compositions was plotted to determine the significance of each composition. Then, all the experiments were repeated with the deployment of a CO_2 diffuser at the sparger to observe the change in time for pH drop.

2.3. Optimization through Surface Response Methodology

Additionally, three factors that affect CO_2 solubility (s), composition (X), pressure (p), and temperature (T) were also studied using three-factor, three-level Box-Behnken design (BBD). Each of these independent factors divided into three different levels as shown in Table 1. To describe the relationship between a set of parameters and output, the regression model was developed in BBD design and can be defined by Equation (1):

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_{ii}^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{ij} X_i X_j \tag{1}$$

where Y: response; β_0: constant-coefficient; β_i and β_{ii}: linear and quadratic coefficients for the terms X_i and X_{ii}, respectively; β_{ij}: coefficients which represent the interactions of X_i and X_j. Then, an analysis of variance (ANOVA) was used to determine whether the models are acceptable for analysis.

Table 1. Levels and ranges of independent input parameters in the Box-Behnken design.

Parameters	Symbols	Level and Range		
		Low	Centre	High
Absorbent	X	Media + NaOH	NaOH	Media + MEA
Pressure, (kPa)	p	100	125	150
Temperature, (°C)	T	20	30	40

2.4. Analytical Methods

Dissolved carbon dioxide (CO_2) in culture media was determined by calculation based on readings obtained from a gas analyzer (Geotech Environmental Equipment Inc., Denver, CO, USA). Readings of pH and temperature were obtained by direct measurement using a portable pH (BOQU®, Shanghai, China) and temperature probe (DPSTAR, Kuala Lumpur, Malaysia). Time was recorded using a stopwatch.

2.5. Model Description and Calculation

Dissolved carbon dioxide (CO_2) in culture media can be calculated using the information given by the gas analyzer. The reading given by the analyzer consists of the composition of air in the headspace. Since the value given by the analyzer is in percentage, some calculations need to be done. The percentage of CO_2 was multiplied by the volume of headspace to get the volume CO_2 in the headspace in m^3. The volume of headspace was constant throughout the experiment. It was set at 2.5×10^{-3} m^3. By subtracting the total value of CO_2 supplied with the one in the headspace, the value of carbon dioxide aqueous, CO_2(aq) can be obtained.

3. Results and Discussion

3.1. Relationship between Carbon Dioxide (CO_2) Concentration and pH

The pH of the absorbent decreased with increasing CO_2 concentration. A slight difference in the lowest value of pH was observed between the absorbent used; sodium hydroxide (NaOH), culture media, and culture media with either NaOH or monoethanolamine (MEA), but the difference was not significant except for MEA which was observed to take a longer time to achieve minimum pH. A significant difference was only in the time taken to achieve the minimum value of pH. The graph of pH against time was plotted for NaOH, MEA, and culture media in Figure 3. The pH decreased with time until the 18 min mark for NaOH and the final pH was 6.91. Meanwhile for MEA and culture media were $t = 60$ min; pH = 8.52 and $t = 8$ min; pH = 6.46 respectively. Finally, culture media with NaOH and MEA were $t = 9$ min; pH = 6.45 and $t = 21$ min; pH = 7.0 respectively.
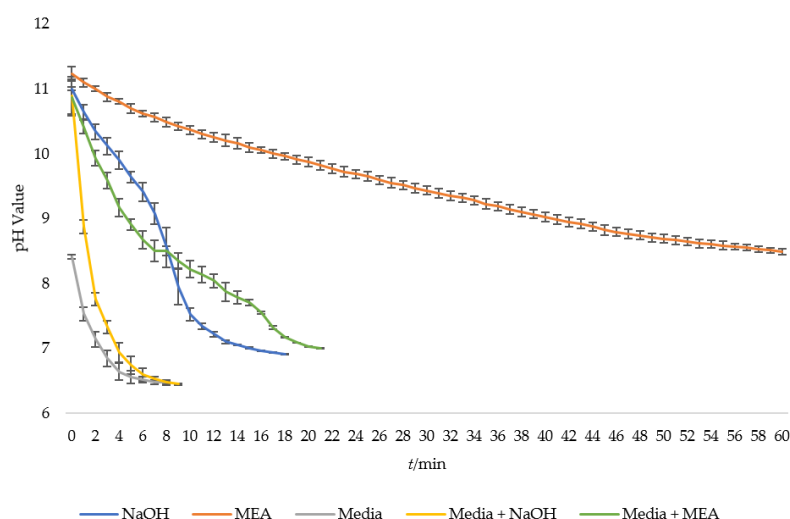

Figure 3. Effect of pH change for five absorbents at $p = 100$ kPa, $T = 20$ °C, with an initial pH of 11 with exception of culture media with an initial pH of 8.5.

Since the behavior of culture media and MEA absorbents do not follow the trend of the other three absorbents, it not suitable to be included in the optimization process (Table 1). The value of pH for culture media cannot be increased without the addition of other substances, thus, the comparison cannot be done as the initial pH value was way lower than other absorbents. For the case of MEA,

the optimization process is used to find the best solution for a range of data, a large difference in data value can resulting in invalid optimization. Because of that, these two absorbents were not included in the optimization analysis. Additionally, the focus was always on culture media with the addition of NaOH and MEA.
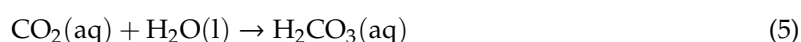
Many factors can affect pH in the absorbent. The decrease in pH value in all absorbents was due to the increase of the concentration of dissolved inorganic carbon such as $CO_2(aq)$, HCO_3^-, and CO_3^{2-} present in CO_2 that been dissolved in said absorbents [15]. Theoretically, NaOH will yield a higher CO_2 solubility than culture media present because the total alkalinity of NaOH is higher due to NaOH is a strong alkaline. The mechanism of CO_2 absorption in NaOH aqueous solution is summarized in the following [16]. All Na^+ and OH^- was ionized in pure water, then aqueous CO_2 reacts with OH^- as expressed in the following equation:

$$CO_2(aq) + OH^-(aq) \rightleftharpoons HCO_3^-(aq) \tag{2}$$

$$HCO_3^-(aq) + OH^-(aq) \rightleftharpoons H_2O(l) + CO_3^{2-}(aq) \tag{3}$$
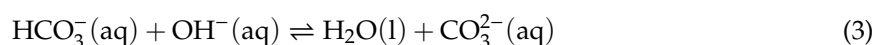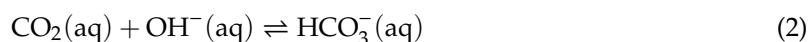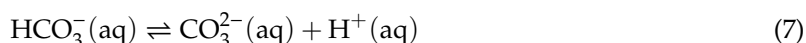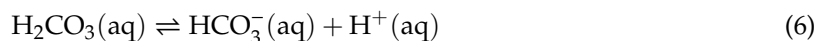
Both equations are reversible reactions with a high effect on pH changes. The reaction is continuous so every $CO_2(aq)$ that was present in the medium will instantaneously be consumed. Due to high alkalinity, reaction Equation (3) was the main reaction occur in the absorbent early on resulting in increasing of CO_3^{2-}, while OH^- is rapidly consumed via both reactions. This explained the reason for the sudden change in pH at the early stage of the experiment. As CO_2 aerated through the medium, OH^- will keep decreasing and CO_3^{2-} keep accumulating. This phenomenon will force a backward reaction of Equation (3) which will accelerate the forward reaction of Equation (2). The pH will keep dropping in this stage. At a certain point in the experiment, pH will stop dropping and remain constant due to the reaction at equilibrium. After all the reactions are at equilibrium, the overall reaction of NaOH with CO_2 can be written as Equation (4).

$$NaOH(aq) + CO_2(g) \rightarrow NaHCO_3(aq) \tag{4}$$
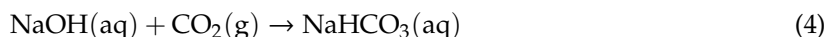
Meanwhile, for the culture media, the phenomenon of CO_2 dissolved can be explained by the reaction between CO_2 and water (because H_2O is present in media). Firstly, aqueous CO_2 will react with water to form carbonic acid [17]:

$$CO_2(aq) + H_2O(l) \rightarrow H_2CO_3(aq) \tag{5}$$

Then the H_2CO_3 can lose one or both of its H^+ to form:

$$H_2CO_3(aq) \rightleftharpoons HCO_3^-(aq) + H^+(aq) \tag{6}$$

$$HCO_3^-(aq) \rightleftharpoons CO_3^{2-}(aq) + H^+(aq) \tag{7}$$

The pH drops in media are due to the released hydrogen ions. However, this equation can operate in both directions depending on the current pH level, working as its buffering system. At a higher pH, this bicarbonate system will shift to the left, and CO_3^{2-} will pick up a free hydrogen ion [18]. But in the system, CO_2 was constantly added to the media, then increasing the value of dissolved CO_2 causing the reaction Equations (6) and (7) forced to be carried out from left to right. This increases H_2CO_3, which decreases pH.

Additionally, based on Figure 2, it shows that culture media with NaOH was the fastest to reach the minimum or equilibrium pH, following by pure NaOH and media with MEA. When the pH reaches its equilibrium point, it indicates that no more CO_2 is being absorbed. With this information, it is concluded that the longer time is taken to reach equilibrium, the higher the CO_2 concentration in absorbent. As expected, culture media capability to absorb CO_2 is weaker than NaOH. However, when MEA was added to culture media, a higher amount of CO_2 was absorbed.

3.2. System Performance with Deployment of Carbon Dioxide (CO₂) Diffuser

One of the main factors in the CO_2 absorption rate is the surface area of the gas–liquid boundary [19]. In the next set of experiments, the factor was been investigated. CO_2 diffuser was mounted at the sparger at the bottom of the reactor where the CO_2 is aerated. This diffuser functions to turn the CO_2 bubble into a more refined form. Parameters used were based on the previous experiments. The result is shown in Figure 4.
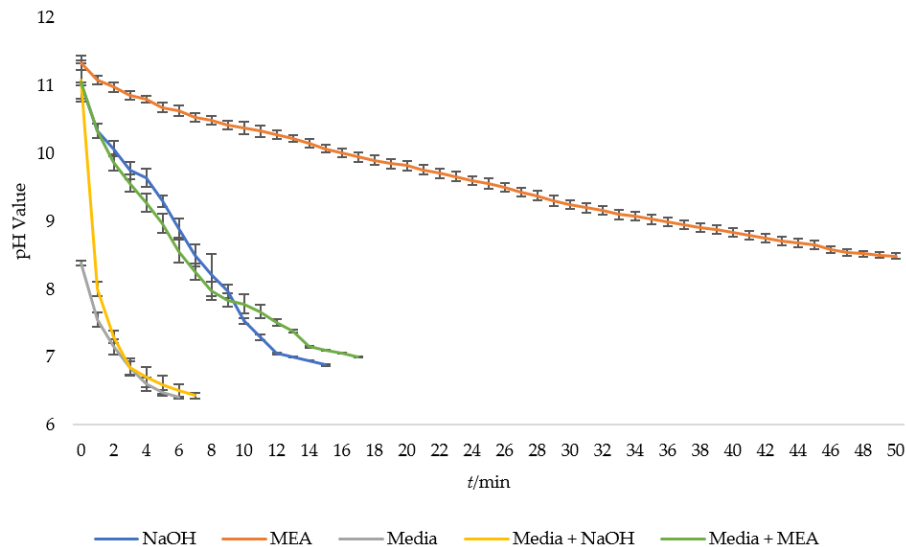

Figure 4. Effect of diffuser on pH change for five absorbents at p = 100 kPa, T = 20 °C, with an initial pH of 11 with exception of culture media with an initial pH of 8.5.

From Figure 4, the addition of the diffuser favorably affected the CO_2 absorption rate by all absorbents. The final pH value were almost identical with previous run, while the time taken for pH to drop for NaOH (pH = 6.88, t = 15 min), MEA (pH = 8.49, t = 50 min), culture media (pH = 6,4, t = 6 min), culture media with NaOH (pH = 6.43, t = 7 min) and culture media with MEA (pH = 7, t = 17 min) were approximately decrease by 20% when using diffuser compared to the previous. By using a diffuser, CO_2 is dissipated into countless small bubbles, which flows in the culture media in the form of a bubbling stream. This process is called atomization. The large number of bubbles scattered in culture media will increase the contact area of gas–liquid that will increase the absorption rate.

A simple relation between the contact surface area and mass transfer rate can be expressed in Equation (8) [20]:

$$m = \int_S j_m dS = S j_m \tag{8}$$

where m, mass transfer, j_m, mass flow, and S, interfacial area, and it is shown that the relationship between mass transfer is directly proportional to the contact area. Additionally, a more detailed mathematical model of absorption rate with other parameters had been derived by Martinez I. et al. [21] using Fick's Law shown in Equation (9):

$$j_g = \frac{q_m}{4\pi r^2} = -D_g \frac{d(w\rho_1)}{dr} \tag{9}$$

where j_g: diffusion mass flux; q_m: mass flow rate; D_g: gas diffusivity; w: mass fraction; ρ_1: density of the liquid used. The assumption is as follows: symmetry is spherical, time-independent, constant

liquid and gas density, and residence time of bubble much smaller than the dissolution time, the factor that affects the absorption rate can be defined by Equation (10):

$$w_\infty(t_v) = w_0[1 - \exp(-Kt_v)], \ \ where \ K \equiv \frac{27v_1 D_g u_g}{2gr_0^4} \tag{10}$$

w_∞: mass fraction before venting; w: gas mass fraction; v: kinematic viscosity of liquid; D_g: diffusion coefficient of CO_2 gas in water; u_g: gas injection speed; g: gravity acceleration; r_0: radius of the bubble. The benefit of this simple mathematical formula is that the relations between different parameters are explicit. Gas diffusion from bubbles to the aqueous phase is measured by w_∞, hence based on this equation, the mass of gas in a liquid is inversely proportional to the fourth power of bubble radius. This experiment only portrays the benefit of a simple diffuser. More complications, especially with made CO_2 diffusers for CO_2 absorption in the reactor can further increase the absorption rate of CO_2.

3.3. Factors Affecting Carbon Dioxide (CO_2) Solubilities in Culture Media

CO_2 is one of the key factors in organic acid fermentation [22,23]. Organic acid had a wide application ranging from preservative agents for food to lab application [24]. Due to its benefit, the production of organic acid is widely studied. As mention earlier, one of the three factors that affect CO_2 solubilities is the composition of media. Many studies focusing on organic acid production have increased the CO_2 availability by the addition of chemicals such as magnesium carbonate ($MgCO_3$), sodium bicarbonate ($NaHCO_3$), or calcium carbonate ($CaCO_3$). Through the addition of such chemicals, the CO_2 solubility is greatly increased. Thus, studies on different chemicals such as sodium hydroxide (NaOH) and monoethanolamine (MEA) are also important.

The theoretical access to CO_2 solubilities in culture media is very limited. The effect of organic solutes on gas solubility can be rather complex. But parameters affecting the solubilities were well-studied. Three parameters affecting CO_2 solubilities are pressure, temperature, and media composition [25]. The effects of these parameters on the solubility of a gas in a pure solvent, expressed in a mathematical model were simplified in Table 2. Based on Table 2, composition (X), pressure (p), and temperature (T) had been identified as the three most important independent parameters affecting CO_2 solubility (s) in absorbent and thus are chosen as the inputs for the design.

Table 2. Mathematical model of effects of pressure, temperature, and composition on gas solubility.

Parameters	Model	Remark	Ref.
Composition	Empirical model of Henry's Law $\log\left(\frac{H}{H_0}\right) = \log\left(\frac{s_{G,o}}{s_G}\right) = \sum_i (h_i + h_G)c_i + \sum_j K_{n,j}c_{n,j}$ whereas H: Henry's constant in solvent mixtures; s_G: CO_2 solubility in solvent mixtures; subscript 0: value at pure solvent (e.g., water); h: ion-specific parameter; $K_{n,j}$: substance specific model parameter; c_i: concentration of ion; $c_{n,j}$: concentration of an organic substance	Solubility depends on ion and organic substance appearing in solvent	[26]
Pressure	Henry's Law: $\rho_{CO_2} = H_0 c_{CO_2}$ whereas p_{CO_2}: CO_2 partial pressure; H_0: Henry's constant; c_{CO_2}: concentration of dissolved CO_2.	Solubility is directly proportional to the pressure	[26]
Temperature	Henry's Law and Van't Hoff equation: $c_{CO_2} = k_H \rho_{CO_2}$ $k_H(T) = k_{298K} \exp\left[-\frac{\Delta H_{diss}}{R}\left(\frac{1}{T} - \frac{1}{298}\right)\right]$ whereas k_h: temperature-dependent Henry's constant; k_{298K}: Henry's constant at 298K; ΔH_{diss}: dissolution enthalpy; R: ideal gas constant	Solubility is inversely proportional to temperature.	[21,22]

3.3.1. Design of Experiment Analysis

In this study, a three-factor, three-level Box-Behnken design (BBD) was used to investigate the effects of composition (X), pressure (p), and temperature (T) the interactions of these factors on the CO_2 solubility (s) in absorbent measured by its concentration. A total of fifteen experimental samples were required for the BBD including three replicated experimental runs using the processing parameters at the center points (Table 3).

Table 3. Actual response of CO_2 concentration at experimental design points.

Exp.	Independent Input Variables [X]			Response [Y]
	Absorbent	$T/°C$	p/kPa	$s/(mol/L)$
1	NaOH	20	100	14.82
2	NaOH	40	100	9.7
3	Culture media with MEA	20	125	18.42
4	Culture media with NaOH	20	125	10.44
5 *	NaOH	30	125	10.04
6	Culture media with MEA	30	100	12.11
7	Culture media with MEA	40	125	12.12
8	NaOH	20	150	16.52
9	Culture media with MEA	30	150	15.76
10	NaOH	40	150	10.4
11	Culture media with NaOH	30	100	8.48
12 *	NaOH	30	125	10.29
13	Culture media with NaOH	30	150	9.56
14 *	NaOH	30	125	9.93
15	Culture media with NaOH	40	125	9.57

* Replicated experimental runs.

The ANOVA analysis is performed as shown in Table 4 to determine the significance and adequacy of the regression models. The Model F-value of 135.76 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise. p-values less than 0.05 indicate model terms are significant and all insignificant model terms had been reduced. Additionally, a graph in Figure 5 shows a good agreement between the actual data and the predicted values from the regression models. The Predicted R^2 of 0.9375 is in reasonable agreement with the Adjusted R^2 of 0.9897; i.e., the difference is less than 0.2. For adequate precision measures of the signal to noise ratio, a ratio greater than 4 is desirable. The ratio of 37.693 indicates an adequate signal. This model can be used to navigate the design space. This result shows that the regression model is statistically significant and adequate for the prediction and optimization of the CO_2 absorption process.

Table 4. Results of ANOVA for the quadratic model of the CO_2 concentration in absorbent.

Source	Sum of Squares	Degrees of Freedom	Mean Square	F-Value	p-Value	
Model	128.74	10	12.87	135.76	0.0001	significant
X-Medium	53.08	2	26.54	279.85	<0.0001	
T-Temperature	42.367	1	42.37	446.76	<0.0001	
p-Pressure	6.35	1	6.35	67.01	0.0012	
XT	9.44	2	4.72	49.78	0.0015	
Xp	2.33	2	1.16	12.28	0.0196	
T^2	14.28	1	14.28	150.60	0.0003	
p^2	2.40	1	2.40	25.34	0.0073	
Residual	0.3793	4	0.0948			
Lack of Fit	0.3112	2	0.1556	4.57	0.1794	not significant
Pure Error	0.0681	2	0.0340			
Corrected Total Sum of Squares	129.12	14				

R^2: 0.9971; adjusted R^2: 0.9897; predicted R^2: 0.9375; Adequate Precision: 37.6933.
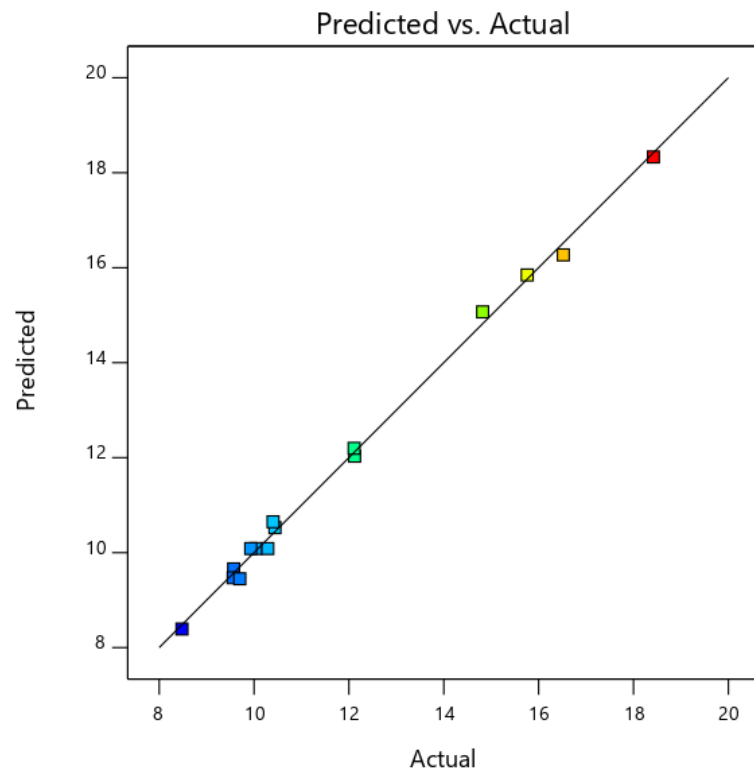
Figure 5. Comparison of actual and predicted results of CO_2 absorption.

Quadratic equations were derived to describe the relationships between the parameters and CO_2 solubility (*s*) as shown in Equations (11)–(13).

NaOH:

$$s = 0.001291(p)^2 + 0.019667(T)^2 - 0.298667p - 1.46100T + 53.38333 \qquad (11)$$

Culture media with NaOH:

$$s = 0.001291(p)^2 + 0.019667(T)^2 - 0.301067p - 1.2235T + 44.5975 \qquad (12)$$

Culture media with MEA:

$$s = 0.001291(p)^2 + 0.019667(T)^2 - 0.249667p - 1.495T + 51.4075 \qquad (13)$$

3.3.2. Individual Effect of Experiment Parameters on Carbon Dioxide (CO_2) Absorption

The significance of each parameter on CO_2 solubility can be illustrated by the perturbation plot in Figure 6, where steep slope and curvature were obtained for both pressure and temperature indicates that CO_2 solubility is sensitive to the parameters. The maximum value of CO_2 dissolved achieved with the addition of NaOH for culture media was lower than what been achieved for MEA. This is due to the MEA's rapid reaction with CO_2 in low partial pressure [27].
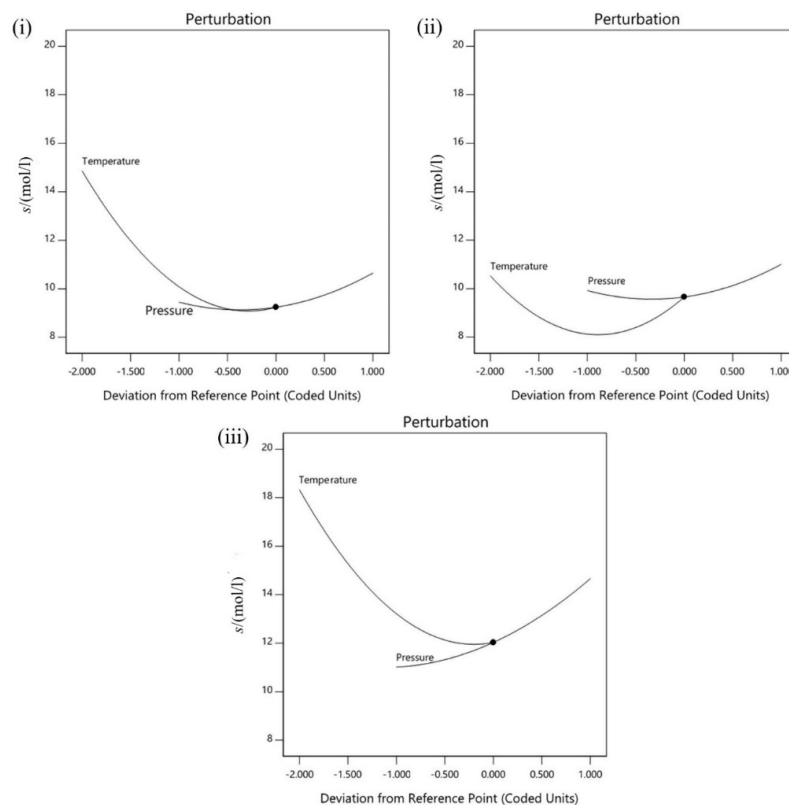
Figure 6. Perturbation plot comparing the response of CO_2 solubility to changes in temperature and pressure in (**i**) pure NaOH, (**ii**) culture media with NaOH, and (**iii**) culture media with monoethanolamine (MEA).

Figure 6 shows the relationship between pressure and final CO_2 concentration which is directly proportional which was described quantitatively by Henry's law shown in Table 2; pressure. External pressure affects the concentration of gas molecules in space. When the partial pressure of the gas above the solution increases, it forces the gas molecules to solute in solution to maintain dynamic equilibrium [28]. Additionally, high temperature demotes CO_2 absorption as opposed to high pressure. Also, the effect of temperature is more significant than the pressure (highest *F*-value). A slight increase in temperature will greatly reduce CO_2 absorption. While to achieve more significant change by pressure, extremely high pressure needs to be applied [29]. The gas dissolves in liquid because of the interactions between its molecules and absorbent. This interaction will release heat when these new attractive interactions form in an exothermic process. Thus, additional heat will produce thermal energy that overwhelms the attractive forces between the gas and the absorbent molecules resulting in less CO_2 dissolved in solution [28].

3.3.3. Collective Effect of Experiment Parameters on Carbon Dioxide (CO_2) Absorption

The *F*-value determine the relative importance of a parameter. From Table 4, the temperature has the highest *F*-value, which is 446.76, showing its importance in CO_2 absorption in this case. Figures 7–9 are three-dimensional response surface and project contour for NaOH, culture media with NaOH, and culture media with MEA, respectively, which show the different experimental parameters and their effects on CO_2 concentration in the absorbent. All graphs derived had curved rather than straight lines, indicating the strong interaction between parameters and the output.
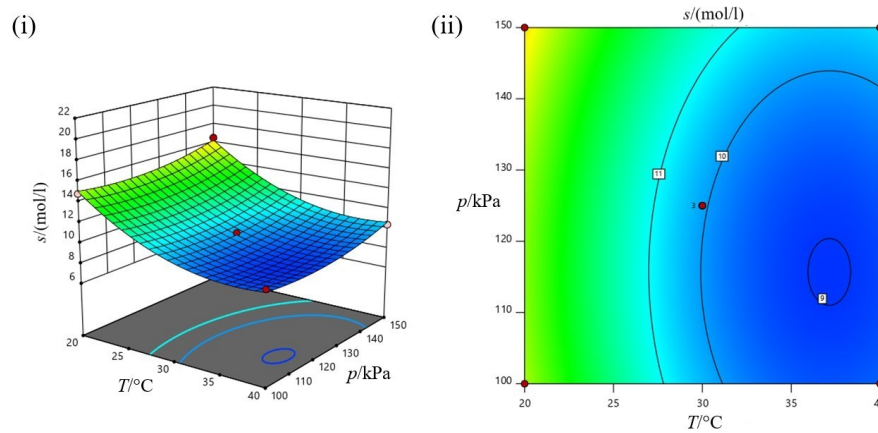
(i) (ii)

Figure 7. Effect of pressure, temperature and their interaction on CO_2 concentration in NaOH (**i**) three-dimensional surface plot; (**ii**) projected contour plot.
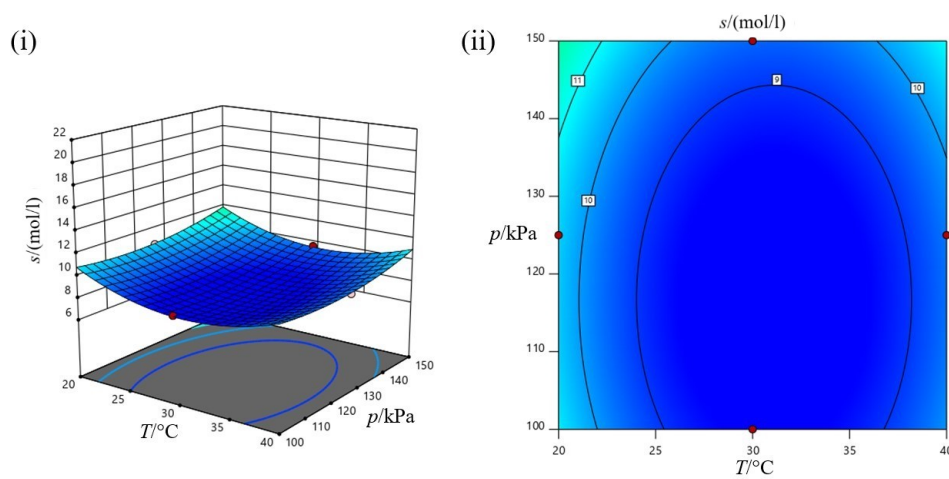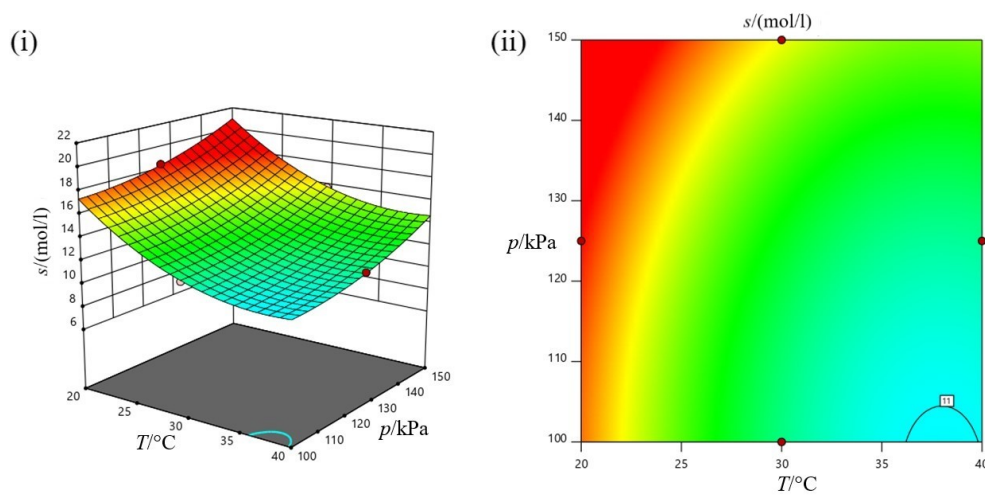
(i) (ii)

Figure 8. Effect of pressure, temperature and their interaction on CO_2 concentration in culture media with NaOH (**i**) three-dimensional surface plot; (**ii**) projected contour plot.

(i) (ii)

Figure 9. Effect of pressure, temperature and their interaction on CO_2 concentration in culture media with MEA (**i**) three-dimensional surface plot; (**ii**) projected contour plot.

3.3.4. Process Optimization

It was observed that carbon dioxide (CO_2) solubility (s) reaches the maximum values under low temperature operating in high pressure. However, there was a certain point in these parameters where a further change in their value will not affect the final CO_2 solubility (s). Therefore, a balance value needs to be established to achieve the maximum result while optimizing the parameters. The purpose of this step is to observe the combination of the independent variable (i.e., absorbent, temperature, and pressure) to get the maximum CO_2 solubility (s) simultaneously. The overall performance of the CO_2 absorption strongly depends on a wide range of experimental conditions. It is crucial to optimize the CO_2 absorption process with multiple inputs and multiple responses. Thus, a series of optimizing results proposed by response surface methodology (RSM) with multiple inputs and multiple responses are listed in Table 5. The optimal CO_2 solubility (s) can be obtained in the following conditions: culture media with MEA as absorbent; 20.483 °C of temperature; 136.728 kPa of pressure. The optimal CO_2 solubility (s) obtained at this condition was 19.029 mol/L. Additionally, a favorable value for each parameter can be chosen from the RSM. For example, the desired temperature is $\approx$ 22 °C, pressure must be around 140 kPa to obtain the optimal CO_2 solubility (s). Though from the optimization process, absorbents other than culture media with MEA was found to be undesirable.

Table 5. Process optimization for CO_2 absorption by RSM.

No.	Absorbent	$T/°C$	p/kPa	$s/(mol/L)$
1	Culture media with MEA	20.483	136.728	19.029
2	Culture media with MEA	20.702	133.270	18.537
3	Culture media with MEA	20.557	132.113	18.529
4	Culture media with MEA	21.951	143.633	18.834
5	Culture media with MEA	20.125	132.344	18.850

4. Conclusions

The present work has investigated the features of sodium hydroxide (NaOH) aqueous solution and culture media to capture carbon dioxide (CO_2) at different compositions, pressure, and temperature. It was observed that the CO_2 solubility increases using monoethanolamine (MEA) compared to NaOH. CO_2 absorption was also favorable at high pressure and low temperature. Improvement of the absorption rate can be achieved by deploying a CO_2 diffuser. Using optimization software, the most optimized condition for the CO_2 absorption process was by using culture media added with MEA, at a pressure of 136.728 kPa operating at 20.483 °C. Besides, capturing CO_2 by using culture media will lead to the production of chemical commodities (such as succinic acid, formic acid, and acetic acid) by microbes that can be useful for industrial usage. This work serves as a base for further research on CO_2 absorption by culture media. Further studies such as the feasibility of this method by using gas mixture instead of pure CO_2 are necessary to demonstrate a more flexible process. The ultimate aim of this work is to produce value-added commodities utilizing CO_2 as the main carbon source with the help of suitable microbes.

Conflicts of Interest: The authors declare that they have no competing interests.

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