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Study of growth kinetics of Antarctic bacterial community for biodegradation of waste canola oil

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ABSTRACT

The growth of bacteria is an important aspect in the biodegradation of pollutants, including hydrocarbons, since hydrocarbon pollution has become an increasingly serious environmental problem in cold regions. The present study investigates factors, such as salinity, nitrogen, yeast extract and waste canola oil that caused inhibitory effect at high concentration on the growth of Antarctic bacterial community known as BS14. Kinetic parameters were calculated using models that consider the substrate's inhibitory effect on bacterial growth; this includes Haldane, Aiba, Teissier and Yano and Koga models. The data were regressed and well-fitted with Teissier model in the inhibitory effect of salt, yeast extract and waste canola oil (WCO) concentrations of –89.908, –70.746 and –57.850, respectively, for the Akaike Information Criterion (AICc) values, whereas the nitrogen source was fitted with Aiba model with AICc value at –84.583. Maximum specific growth rate (μ_{max}) for each factor exhibited various speeds in cell growth rate where the μ_{max} for the inhibition of growth by salt, nitrogen, yeast extract and WCO were at 1.004, 0.131, 1.005 and 0.544 h^{-1}, respectively. The growth rate of Antarctic bacterial community BS14 was evaluated through non-linear regression model and the concentrations of substrate inhibition were identified.

Keywords: Kinetic model; Antarctic community; Salinity; Nitrogen source; Yeast extract; Waste canola oil

1. Introduction

Antarctic is known as an isolated, confined, protected continent with the coldest, driest, windiest hence with the most extreme weather environment in the world [1]. Antarctic has important environmental, scientific and intrinsic values worthy of protection for the future of the world [2]. It is known as the cleanest land on the earth, making it a perfect place for judging the spread of global pollutants and is a sensitive indicator of the worldwide revolution [3]. Although Antarctica is often known to be pristine land, the human activities from global (industrialised sites)

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and local sources (waste incineration plant, fuel consumption, sewage production, tourism and transportation) have caused pollutions in Antarctica for the past half-century [4,5]. Oil pollution is one of the serious issues in Antarctica [6]. Oil wastes such as cooking oil, lubricating oil, engine oil and automatic transmission fluid oil were generated up to 1.4 tons annually from Maitri station [7]. Vegetable oils are important for cooking in Antarctic station; most of the kitchens in Antarctic station base used canola oil. The used vegetable oils cannot be released to the Antarctic environment because it poses environmental problems. Vegetable oil would coat the animals and suffocate them by oxygen depletion and in the worst case possibly could destroy the habitats and food supplies of animals [8,9]. Most of the organisms can suffer physical injuries such as the loss of mobility and smothering. Generally, the oil will affect the marine animals such as sea otters since this animal depend on their clean fur to swim, at the same time oil also may cause the inhibition of the development of unborn turtle during breeding season through contamination of eggs [10,11].

This pollutant could persist in the environment for many years, especially in a cold environment such as Antarctica [6,12–15], therefore waste cooking oil need to be transported out of Antarctica. The transportation of the waste vegetable oil from station base through Antarctic cruise ship for waste disposal could increase the possibility of the hydrocarbon spills to the environment through leakage [16]. It was also reported that during 1967 until 2003, there were accidents and incidents during travel to the Antarctic such as aircraft crash, ship grounding and oil spoil, which also causes oil spill to the environment.

Bioremediation using microorganisms is one of the most cost-effective and eco-friendly [17–20] techniques since chemical or physical remediation could lead to secondary pollution especially when there is a continuous generation of pollutants due to anthropogenic activities [21]. Bioremediation of hydrocarbons, oil and grease using bacteria have been reported such as *Aeromonas hydrophila*, *Bacillus cereus*, *Serratia marcescens* and *Rhodococcus* sp. [22,23]. All these bacteria reported were able to breakdown the vegetable oil due to their production of hydrolytic enzymes, including lipase, which allows the remediation process to occur. In bioremediation technique, mix or consortium of microorganisms have been studied and claimed to be more effective; yielded better result in degradation compared with the single culture [24]. This may be due to the synergistic effect among the members of a specific consortium [25]. Moreover, the survival of bacteria is better in a group of community [26] since different types of bacteria were able to produce different catalytic enzyme functions with different substrate specificity and enzyme stability that will help the bacteria to survive especially in unfavourable conditions [27].

Modelling of biological remediation process can be a very useful tool for determining the requirements of microorganism's biomass inputs, the duration for the process to be achieved and the effects of environmental conditions including the influence of soil on remediation efficiency [28,29]. Non-linear models are difficult to specify and estimate when compared with a linear model. However, for prediction purpose, it is very important to estimate the relationship between the specific parameters properly [30]. The estimated parameters can be calculated from the data through non-linear form of the model [31], and it is the best way to predict the growth rate using different models suggested by previous researchers. The parameters that can be obtained through non-linear regression models include specific growth rate, maximum growth rate, half-saturation constant, inhibition constant, substrate concentration, critical substrate concentration above which growth completely stops and the exponent representing the impact of the substrate to μ_{max}, respectively [32]. For example, Monod type model predicts the presence of critical inhibitor concentration above which cells cannot grow, and that the constants of the Monod equation are the functions of this limiting inhibitor concentration [33]. This model equation was proposed similar to the Michaelis–Menton representation of enzyme kinetics [34].

The determination of growth kinetics of microorganisms on environmental pollutants has been reported in many past studies using crude oil, hydrocarbons, phenol, atrazine and toluene in single culture and consortium [35–39]. Nevertheless, only few studies have been reported in growth kinetics by modelling several inhibition effects during biodegradation of vegetable oil using Antarctic bacterial community. The growth of microorganisms are affected by chemical, physical and nutritional factors, where optimal growth condition is vital for the bacteria to perform their metabolic activities; showing the importance of studying the bacterial growth kinetics. Several studies have testified on the effects of temperature, salinity, yeast extract, nitrogen and carbon source on the growth rate of the mesophilic microorganisms such as *Zygosaccharomyces rouxii*, *Shewanella gelidimarina*, *Enterococcus faecalis* RKY1, *Halomonas campisalis* and *Prorocentrum donghaiense* [40–45].

The aim of this study was to investigate the kinetic growth rate of Antarctic bacterial community in biodegradation of WCO. Different factors affecting the growth of the Antarctic microbes in biodegradation of waste vegetable oil was determined. The growth kinetics were studied using different types of non-linear model to investigate the best-fitted model for the bacterial community for bioremediating WCO in different concentrations of salt, nitrogen source, yeast extract and WCO.

2. Materials and methods

2.1. Medium for growth

Antarctic bacterial community known as BS14 capable of degrading WCO was selected from soil collected from Bernardo O'Higgins Riqueleme, Chilean Antarctica Base. The medium used for the growth of this bacterial community was a modified mineral salt medium (MSM) that composed of (g/L): 7.74 g of Na_2HPO_4, 2.913 g of NaH_2PO_4, 0.5 g of $MgSO_4{\cdot}7H_2O$, 0.01 g of $CaCl_2{\cdot}2H_2O$ and 1 g of $(NH_4)_2SO_4$ in distilled water and the medium was altered at pH 7 [23]. After 2 d incubation of culture in nutrient broth at 10°C, the cells were harvested by centrifugation at 7,000 × g for 10 min. The pellets were washed twice with 1X PBS (pH 7.4) and adjusted to OD_{600} = 1.0 ± 0.1 prior to conducting the experiment of the determination of the growth kinetics in MSM. WCO was obtained from Antarctic base station.

2.2. Growth parameters

In the present study, all factors were varied at seven parameters point; saline range of 0.0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0% (w/v), concentration of nitrogen ranging from 0.0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 g/L, the amount of yeast extract that was differed at range of 0.00 to 1.25 g/L and concentration of substrate starting from 0.5, 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0% (v/v). All 250 mL flasks containing 50 mL of MSM were cultured with 1 mL of bacterial community and 1% (v/v) of WCO was added in each flask, then incubated at 10°C on 150 rpm orbital shaker and routinely measured the bacterial growth using spectrophotometer for 7 d. All experiments were carried out using one-factor-at-time (OFAT) approach by maintaining the parameters that showed the highest bacterial growth turbidity at OD_{600nm} for the subsequent factors. The analysis of variance was analysed using GraphPad Prism software (Version 5) through one-way ANOVA of Tukey's test.

2.3. Kinetics modelling

The specific growth rate for each factor was obtained and modelled using different types of non-linear regression method. The predictions of the model were varied with each other since they proposed different situations for the enzyme activity in the presence of substrate. The amount of substrate (pollutants) has an important role in optimising performance of the bioremediation, where these modelling approaches were able to predict the substrate consumption by analysing from the bacterial growth behaviour [46]. Determination of intrinsic growth kinetic parameters can be modelled based on several non-linear regression modelling of the bacterial growth rate profile using substrate inhibition models such as Yano, Teissier–Edwards, Aiba and Haldane models. The formulae for the above model are shown in Table 1, where μ, μ_{max}, K_s, K_i and S are specific growth rate (h^{-1}), maximum growth rate (h^{-1}), half-saturation constant (mg/L), inhibition constant (mg/L) and substrate concentration (mg/L) [32,47]. The values of the specific growth rate coefficient at each initial contaminant concentration can be obtained by plotting InX (bacterial numbers) vs. time.

2.4. Statistical analysis

The non-linear regression models were fitted to the specific growth rate using CurveExpert Professional software (Version 2.6.3). The best-fitted model was statistically assessed through various parameters generated from the software. These included the coefficient of determination (R^2), root-mean-square error (RMSE) and Akaike Information Criterion (AICc) [52]. Small values of RMSE and AICc were expected, which are highly accurate for the models with the equations. At the same time, the accuracy bias (AF) and bias factor (BF) were calculated to determine the quality of fit between the models regressed. Using Eqs. (1) and (2), the AF and BF were calculated for testing the goodness-of-fit of the models [53].

$$\text{Bias factor} = 10^{\left(\sum_{i=1}^{n}\log\frac{\text{Pd}_i/\text{Ob}_i}{n}\right)} \quad (1)$$

$$\text{Accuracy factor} = 10^{\left(\sum_{i=1}^{n}\log\frac{|\text{Pd}_i/\text{Ob}_i|}{n}\right)} \quad (2)$$

where Pd indicates the predicted values and Ob indicates observed values. All experiments were carried out in triplicate and the data obtained are presented as ± standard mean error (SEM).

3. Results and discussions

Antarctic bacterial community collected from soil sample at the Chilean station was tested on its ability to grow on waste canola oil (WCO). In order to determine the best-fitted kinetic model with the specific growth rate of the bacteria, the effect of different concentrations of salinity, nitrogen, yeast extract and WCO was tested on the growth of BS14 culture.

Table 1
Kinetic models to study the effect of substrate on growth rate

Model	No. of parameters	Equation	Refs.
Aiba	4	$\mu_{\max\frac{S}{K_{S+S}}\exp\left(-\frac{S}{K_i}\right)}$	[48]
Haldane	3	$\mu_{\max\frac{S}{S+K_s+\frac{S^2}{K_i}}}$	[49]
Teissier–Edwards	3	$\mu_{\max}\left[1-\exp\left(-\frac{S}{K_i}\right)-\exp\left(\frac{S}{K_s}\right)\right]$	[50]
Yano	4	$\frac{\mu_{\max S}}{S+K_s+\left(\frac{S^2}{K_1}\right)\left(1+\frac{S}{K}\right)}$	[51]

3.1. Effect on different concentrations of salinity, nitrogen, yeast extract and WCO on growth of Antarctic bacterial community (BS14)

The sequence of each parameter was selected by knowing the important factors that affect the bacterial growth to survive in different conditions, especially for bioremediation purposes. Salinity is one of the physicochemical factors affecting the bioremediation since it is a crucial factor to consider first. The water availability in the environment is necessary for the chemical reactions in the cell and for the transport of nutrients into and wastes out of the cell [54]. The use of NaCl helps in controlling the water activity for the bacteria to grow. The media was then varied with different concentration of nitrogen and yeast extract. The availability of nitrogen and yeast extract stimulates the bacterial cell growth as these compound work as nutrients and energy to build proteins and structural membranes of the bacteria. Each optimum condition obtained from the previous factors was used in the last factor, as it was predicted for the bacteria able to survive in a high amount of WCO and degrade the oil.

Different basic growth models were observed for each factor's parameters. The bacteria could grow and survive in different concentrations of salinity, both in hyposaline or hypersaline environment due to the ability of the bacteria to have dissimilar osmotic stress response. The growing community of BS14 in low saline showed an increased number of cells for non and low-saline media and low growth in high salinity of the media ($p < 0.05$; Fig. 1a). The log phase of this bacterial community at 0.00% and 0.25% of NaCl was longer compared with the log phase of this bacterial community at the condition of high salt concentration, where increased salinity had caused the growth of the bacterial community to start becoming consistent from day 3. However, the growth of the bacteria in different concentrations of nitrogen source exhibited different pattern of bacterial growth (Fig. 1b). Ammonium sulphate ($(NH_4)_2SO_4$) was utilised as the nitrogen source by the BS14 Antarctic bacterial community to grow. At 0 g/L of $(NH_4)_2SO_4$, the bacterial community had a long lag phase (6 d). The adaptation of the bacterial community in the medium containing the 1% WCO (as carbon source) but without the nitrogen source was slow. This shows the importance of nitrogen source for the growth of the bacterial community. On the other hand,bacterial growth increased steadily from day 3 to day 5 at 3.0 g/L of nitrogen concentration and slightly faster than at 2 to 2.5 g/L when it reached at day 7 and the log phase for the 0.5 g/L of nitrogen displayed high-steep slopes compared with other concentrations (Fig. 1b). This finding was in line with previous studies, whereby Antarctic bacteria have been reported for high bacterial growth and degradation of xenobiotics in the presence of $(NH_4)_2SO_4$ [39,55–57].

The growth of the bacterial community in different concentration of yeast extract was determined. Without yeast extract, the growth of bacteria was slow (Fig. 1c). However, bacterial growth increased rapidly as the yeast extract was added into the medium. During 6 and 7 d incubation time, there was no significant difference ($p > 0.05$) in the bacterial growth in the medium containing 0.75–1.25 g/L of yeast extract. Similarly, the *Actinobacillus* sp. P3(7), *Bacillus subtilis* 3KP, *Pseudomonas putida* TI(8) and *Micrococcus* sp. LII(61) bacteria could grow rapidly in the hydrocarbon MSM enriched with yeast extract [58]. Yeast extract promotes the cell growth and induces the biodegradation of pollutants, especially hydrocarbons, where it serves as vitamin, mineral and nucleic acid for the bacteria [59]. On the other note, the presence of yeast extract seems to induce the growth of the bacterial community in the presence of WCO as contaminant. At low WCO concentrations, the bacteria were able to grow rapidly within 72–96 h, where the turbidity of the bacteria was approximately ~8.0 at OD600 nm for both 0.5% and 1.0% of WCO compared with the growth of the bacteria at high WCO concentrations (Fig. 1d). This could be attributed to the toxicity of the oil itself that led to bacterial growth inhibition and disturb some catalytic enzymes, including the responsible enzymes in degrading WCO [60]. The high growth of bacteria could also induce the responsible bacteria to degrade WCO and help in biodegradation of WCO. Based on the previous study, at 0 concentration, yeast extract exhibited 23% degradation of diesel oil and rapidly increased to 60% degradation in the presence of 13 mg/L of this additional substrate [61]. The growth of the bacterial community started to decrease at 144–168 h, especially at high concentrations of WCO. However, at 168 h incubation time, there was no significant difference ($p > 0.05$) of the bacterial growth between all the concentrations of WCO.

As OFAT approach was carried out, the experimental results in this study in Figs. 1c and d show high bacterial growth compared with Figs. 1a and b. The highest parameter in previous factors was used for the next factors; hence the bacterial growth will keep increasing from one factor to another. Fig. 1 shows that the most significant factor that affect the bacterial growth was yeast extract as the turbidity of the growth increased five times. As mentioned before, yeast extract helps in bacterial growth with the presence of oil and reduce the inhibitory effect of the oil to the bacterial growth. Hence, the highest bacterial growth in 1% of WCO media was at 0% of NaCl, 1 g/L of $(NH_4)_2SO_4$ and 1 g/L of yeast extract. These optimal conditions were used in the media consisting of different concentrations of WCO and exhibited the ability of the bacteria to survive and grow in the high concentration of WCO media.

Generally, in bacteria growth, an organic pollutant such as WCO is used as a source of carbon and energy. The availability of WCO as nutrient encourages the bacterial growth and allows biodegradation process to occur. Based on Fig. 1, the bacteria were able to survive and continuously grow until day 7 incubation time. This showed that the bacterial community could have consumed WCO and that promoted the growth of the bacterial community. According to the previous study, most of the bacteria showed high bacterial growth and better performance on biodegradation of pollutants; this may be because of the bacterial consumption of the pollutants (such as diesel and vegetable oil) as their nutrient sources that can act as carbon source while glyphosate and polyacrylamide act as nitrogen sources [26,47,62,63].

3.2. Modelling the bacterial growth kinetics

Based on our findings, most of the factors that affect the growth of bacteria have been shown to have an inhibitory

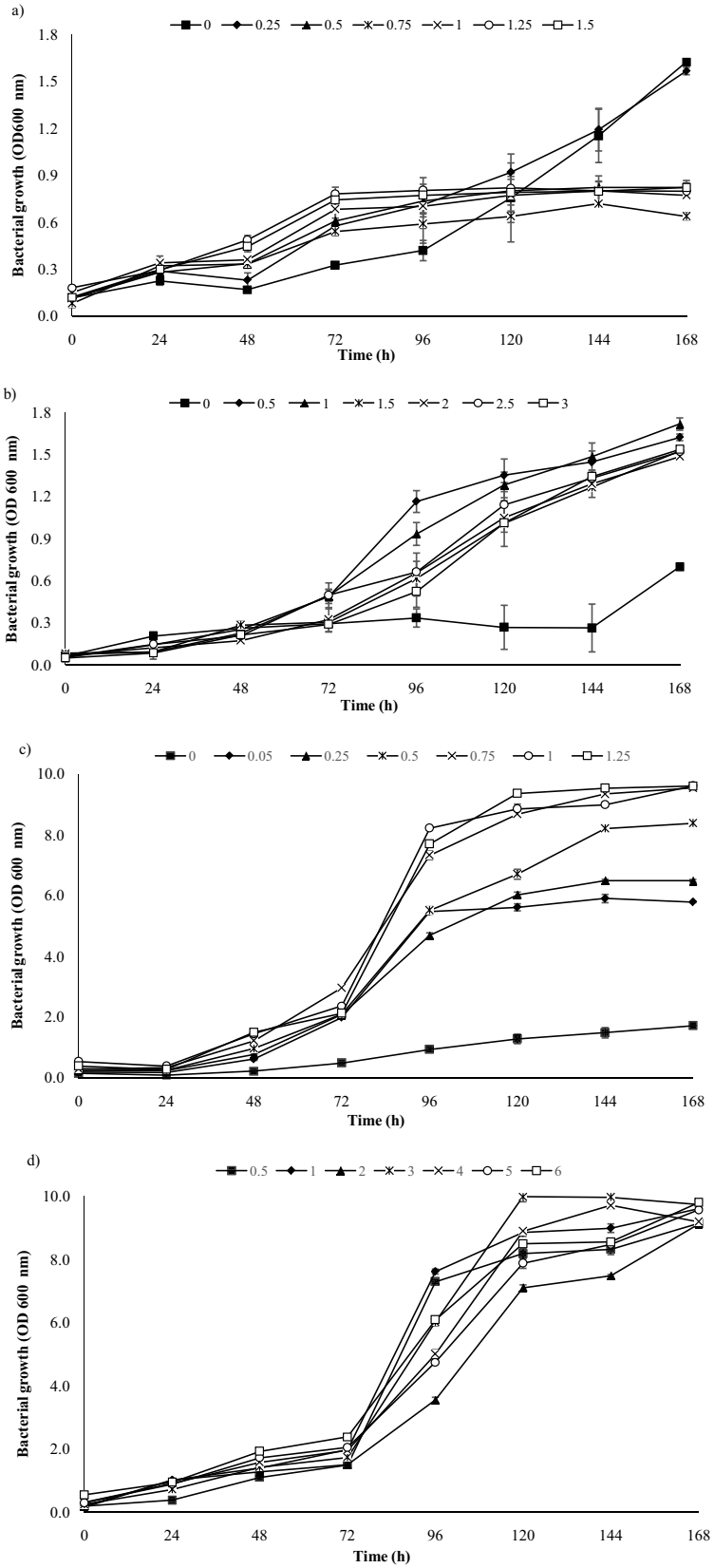

Fig. 1. Effect of varying the concentration of (a) NaCl by fixed $(NH_4)_2SO_4$ at 1 g/L; yeast extract at 0 g/L; 1% of WCO, (b) $(NH_4)_2SO_4$ by fixed NaCl at 0%; yeast extract at 0 g/L; 1% of WCO, (c) yeast extract by fixed NaCl at 0%; $(NH_4)_2SO_4$ at 1 g/L; 1% of WCO and (d) WCO by fixed NaCl at 0%; $(NH_4)_2SO_4$ at 1 g/L; yeast extract at 1.25 g/L on the Antarctic bacterial community growth for 7 d incubation at 10°C.

effect at higher concentration of substrate. Data from Fig. 1 were used to plot the specific growth rate and predicted using different types of kinetics model equation that were proposed to have an inhibitory effect at high concentration of the substrate. Kinetic profiles were successfully fitted using non-linear regression models; Aiba model 1968, Haldane model 1930, Teissier model 1942, and Yano and Kago model 1969, which are widely used in predicting maximum specific growth rate in bioremediation of xenobiotics. It can be observed that the experimental (EXP) growth rate value of the Antarctic bacteria was at finest fit with Teissier model for all factors (Fig. 2). Teissier model allows the prediction of substrate inhibition at high substrate concentration [33]. Mathematical models, including Teissier model, can describe the rate of bacterial growth that was effected from other nutritional and environmental factors which incorporated with the inhibition of the bacteria in the presence of contaminants as substrate [44,64]. The bacterial growth normally will inhibited in unfavorable condition (pollutant presence), however the optimum condition for the bacteria to grow including the availability of nutrients and environment such as osmotic stress can developed the ability of the bacteria to survive. Nevertheless, the accuracy in determining the best-fitted graph can be obtained through statistical analysis on several statistical measurements, including R^2 and RMSE values as stated in Table 2.

In modelling the growth kinetics on the effect of NaCl in the media, Teissier model generated high R^2 value with the smallest value of RMSE, which was at 0.967 and 0.00130, respectively (Table 2). Under other conditions in different concentrations of $(NH_4)_2SO_4$, all models showed a high value of R^2; more than 0.900 and low value of RMSE and AICc according to Table 2. The finest fit for this condition was achieved by regressing the EXP data with predicted value through Aiba model followed by Teissier model. When comparing these two models, the best regression chosen via the AICc and F-test was the Aiba model and the likelihood that Aiba is the better model was 93.39%. Moreover, Aiba model was found to be a perfect equation to demonstrate ammonium as an inhibitory substrate of nitration process in immobilised biomass system [65]. Originally, the Aiba model is an empirical correlation between simulated growth kinetics data predicted by the model with the influence of substrate inhibition [48]. The product biomass is inhibitory to the metabolic activity of the cells; however, the modified Aiba model takes into consideration the total inhibition concentration [66]. Nevertheless, Aiba and Teissier models showed a statistically insignificant difference for the R^2 and RMSE values ($p > 0.05$). Accuracy (AF) and bias factor (BF) specify a perfect agreement between observed and predicted generation time. Larger than 1 indicates less accuracy on the average estimate for AF, while Mellefont et al. [53] mentioned in the situation of BF > 1, the model predicts generation times longer than observed and BF < 1 showed shorter generation time than observed since the BF is measured to determine and the relative average deviation of predicted and observed generation times (Table 2).

The kinetics growth that affected yeast extract showed higher R^2 value for Teissier model than Aiba model, which showed R^2 equal to 0.989 and 0.977, respectively. The statistical analysis showed different best regression chosen via AICc and F-test, where through AICc test, Teissier was chosen for the best regression, whereas Aiba model was chosen from the regression via F-test. Regardless of these two models, the goodness-of-fit was measured and generated Teissier was the better model with 60.49% than Aiba model. In different concentrations of WCO, the EXP data of growth rate fitted to the models, and the highest value of R^2 was seen on Yano and Koga model, which was at 0.876. However, the value of predicted error was smaller for Teissier model compared with Yano and Koga model. The Teissier model was chosen as a better model because of the probability of 91.49% than non-linear regression models tested.

All the models predicted the maximum specific growth rate per hour (μ_{max}) and half-saturation coefficient (K_s), as well as minimum and maximum estimates for the range of

Table 2
Statistical analysis of kinetic models for various models in different conditions of the media

Factors	Model	R^2	Adj. R^2	RMSE	AICc	SSE	AF	BF
Salinity	Aiba	0.885	0.862	0.00218	–76.246	0.00238E-02	0.996	1.003
	Haldane	0.769	0.633	0.00356	–85.341	0.0035	0.999	1.001
	Teissier	0.967	0.951	0.00130	–89.908	0.0068E-04	1.001	0.998
	Yano and Koga	0.859	0.753	0.00352	–72.718	0.0037E-02	0.997	1.003
Nitrogen source	Aiba	0.959	0.951	0.00230	–84.583	0.0027E-02	1.001	0.999
	Haldane	0.903	0.853	0.00399	–74.323	0.0064E-02	1.003	0.997
	Teissier	0.952	0.928	0.00279	–79.29	0.0031E-02	1.000	1.000
	Yano and Koga	0.946	0.879	0.00512	–71.386	0.0079E-02	1.002	0.998
Yeast extract	Aiba	0.977	0.972	0.00658	–69.894	0.0022E-01	0.987	1.013
	Haldane	0.841	0.757	0.0194	–52.278	0.0015	0.996	1.003
	Teissier	0.989	0.983	0.00513	–70.746	0.0011E-01	0.987	1.013
	Yano and Koga	0.964	0.928	0.0106	–55.664	0.0033E-01	0.990	1.010
WCO	Aiba	0.782	0.739	0.0131	–60.300	0.0085E-01	0.987	1.013
	Haldane	0.657	0.500	0.0221	–52.924	0.0020	0.996	1.004
	Yano and Koga	0.876	0.741	0.0130	–53.101	0.0051E-01	0.990	1.010

reasonable curve fits; however, Aiba models did not predict the substrate inhibition constant (K_i). Table 3 shows the parameters generated for the best-fitted model for each factor where NaCl, yeast extract and WCO concentrations were best fitted with Teissier, while Aiba model showed the finest fit with the kinetic growth of bacteria affected by different concentrations of nitrogen source. The effects of NaCl concentration on specific growth rate (μ) can be seen decreasing in the high concentration of NaCl since the Antarctic bacterial community BS14 was collected from the soil sample. In nature, this community cannot grow at high concentration of NaCl. It has been reported that several Antarctic bacteria, especially soil sample bacteria, prefer low salinity for them to grow. Examples of Antarctic bacteria that are able to grow in low saline and have a high growth rate in this condition are *Rhodococcus* sp. strain AQ5–07 and *Arthrobacter* spp. [67,68]. It was predicted that maximum specific growth rate (μ_{max}) of 1.004 (range 1.000 to 1.008) h^{-1} for growth of the bacteria and half-saturation coefficient (K_s) was shown to be 0.275 (range 0.214% to 0.337%). At the same time, the substrate inhibition constant (K_i) predicted for this model was at 0.265 (range 0.204% to 0.326%). The range values in brackets indicated the minimum and maximum estimates for curve fit model. The μ_{max} in biodegradation of perchlorate by unidentified bacterial community in a range of 0% to 1% NaCl concentration was reported by Park and Marchand [69], which at 0.0286 h^{-1} with range of 72.8 to 57.6 mg L^{-1} for K_s value. Basically, half-saturation coefficient (K_s) plays an important role in defining the concentration at which kinetic rate will become substrate-limited to half of the maximum rate as the growth rate is strongly dependent on the K_s at low substrate concentration [70].

Further study on the growth kinetics rate on the influence of different concentration of $(NH_4)_2SO_4$ was carried out and discovered that Aiba the best-fitted model with maximum specific growth rate (μ_{max}) at 0.131 (range 0.105 to 0.157) h^{-1}, half-saturation coefficient (K_s) given at 0.813 (range 0.636 to 0.990) g/L for the growth of the bacteria and the substrate inhibition constant (K_i) displayed at 0.688 (range 0.662 to 0.886) g/L (Table 3). *Prorocentrum donghaiense* growth was best fitted with non-inhibitory effect model (Monod), where the μ_{max} and K_s value on nitrogen uptake of ammonium ranging from 0.5 to 500 μmol N L^{-1} in concentration was at 0.83 d^{-1} and 0.51 μmol N L^{-1}, respectively [44]. The μ_{max} value was higher compared with the Antarctic bacterial community of BS14 with the addition of WCO that also can cause the inhibition of bacteria to grow. The high concentration of nitrogen source lowered the growth rate of the bacterial community. As reported by Metsoviti et al. [71], nitrogen concentration can affect the growth rate of their algae identified as *Chlorella vulgaris*, where at 400 to 800 mg N/L, the rate started to decrease. However, there was a study using a pure culture of *Escherichia coli* that showed a decline in growth rate under nutrient limitation (NH_4Cl), suggesting that this nitrogen source is not a toxic or inhibitory substance towards the bacteria [72]. It can be assumed that the pure bacteria and bacterial community used in this study showed different kinetics models between each other.

Yeast extract can cause an inhibitory effect to the bacterial growth since it also can also serve as a carbon source for the microorganisms. The high concentration of substrate, especially when acting as a carbon source, can cause the growth rate of bacteria to become slower. Moreover, the medium used in this study was introduced with 1% of WCO as a carbon source (pollutants). However, the maximum specific growth rate (μ_{max}) value was predicted through Teissier model at 1.005 (range 0.992–1.018) h^{-1}, while half-saturation coefficient (K_s) and substrate inhibition constant (K_i) values were assumed as 0.245 (range 0.216–0.274) g/L and 0.183 (range 0.157–0.209) g/L, respectively. The presence of yeast in the degradation of chlorobenzoic acids showed inhibition effects by *Alcaligenes* sp. CPE3 and not in the absence of yeast extract [73]. The value of degradation rate (μ) was 30% higher in the absence of yeast than in its presence, which the maximum specific degradation rate (μ_{max}) value were 0.290 and 0.222 h^{-1} for the absence and presence of yeast, respectively. Nevertheless, different condition were reported by Armenante et al. [73], where high μ_{max} in both presence and absence of yeast extract in biodegradation of chlorobenzoic acids. This can happen because yeast extract are known as inducer for the bacteria in degrading hydrocarbons.

A similar pattern was shown in the bacterial growth rate affected by different concentrations of WCO. Besides, the maximum specific growth rate (μ_{max}) was at 0.997 (range 0.961–1.032) h^{-1}. At high concentration of WCO (3%), the growth rate of the bacteria became slow (less than 1.000) h^{-1} (Fig. 2d). The high concentration of WCO could be toxic to the bacteria that it impeded the growth. This condition also has been proven in many studies on kinetics biodegradation of pollutants by bacteria including the community bacteria, where all the pollutants act as substrate inhibition at high substrate concentration [74–77]. The finest Teissier model for this factor demonstrated 1.678 (range 0.885% to 2.471%) half-saturation coefficient (K_s) and pointed the substrate inhibition constant (K_i) of 1.437 (range 0.695–2.179) %. Previous study by Sadouk-Hachaichi et al. [78] showed the growth of six strains isolated from oily sludge; the

Table 3
Specific growth kinetic parameters acquired for the best-fitted model for the factors that affected Antarctic bacterial community (BS14) growth rate

Factors	Unit factors	μ_{max}	K_s	K_i
NaCl	% w/v	1.004 (1.000–1.008)	0.275 (0.214–0.337)	0.265 (0.204–0.326)
$(NH_4)_2SO_4$	g/L	0.131 (0.105–0.157)	0.813 (0.636–0.990)	0.688 (0.662–0.886)
Yeast extract	g/L	1.005 (0.992–1.018)	0.245 (0.216–0.274)	0.183 (0.157–0.209)
WCO concentration	% v/v	0.997 (0.961–1.032)	1.678 (0.885–2.471)	1.437 (0.695–2.179)

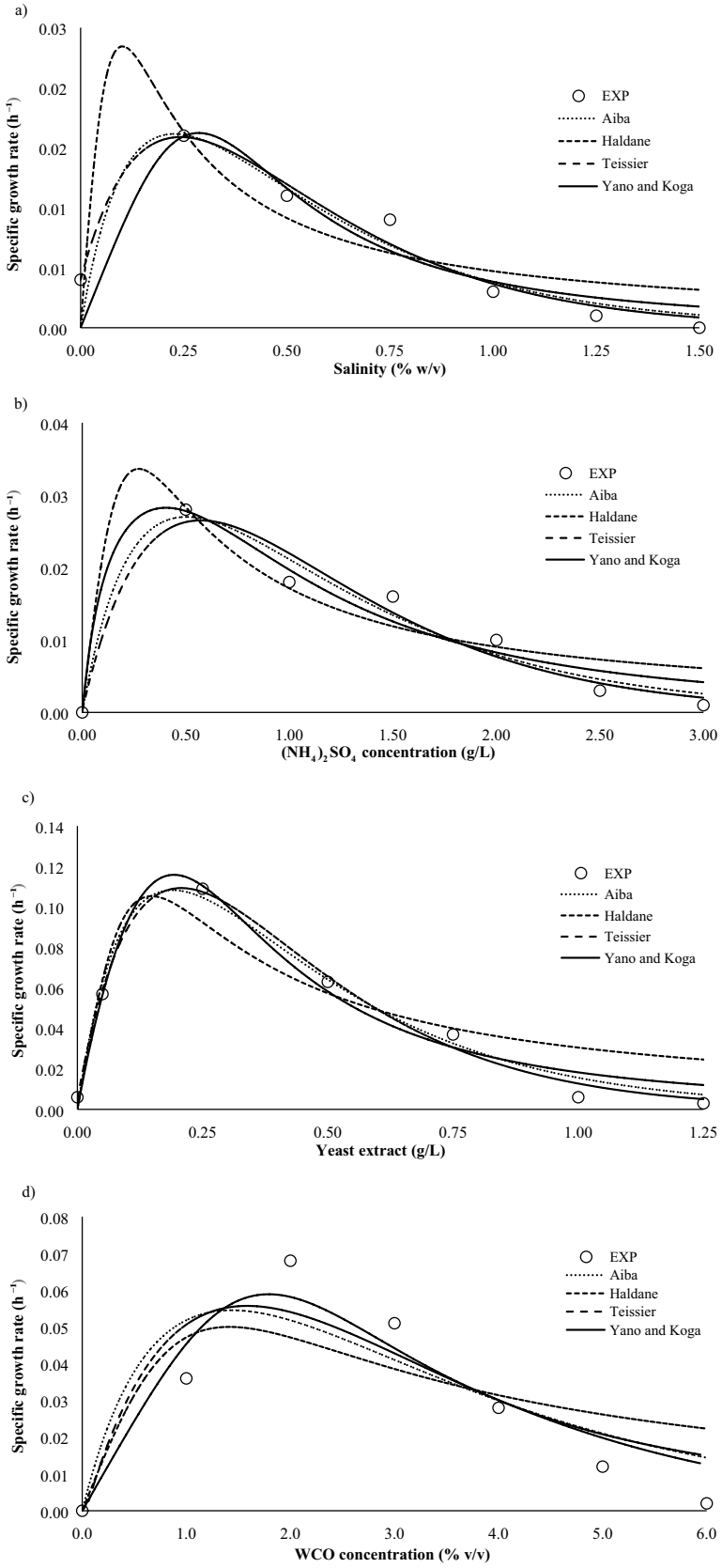

Fig. 2. Predicted specific growth rate of the Antarctic bacterial community (BS14) at different concentrations of (a) NaCl, (b) $(NH_4)_2SO_4$, (c) yeast extract and (d) WCO with different non-linear regression models.

obtained data were regressed with the Andrews inhibitory model and revealed the μ_{max} value and K_s value at 0.535 h^{-1} and 18.68 g/L, respectively. The bacterial community were grown with diesel oil at initial concentrations varying from 8.4 to 84.0 g/L. As shown in Table 3, all factors with best-fitted model showed high value of K_s than K_i ($K_s \leq K_i$), which indicate the possibilities of the factors tested on the growth of the bacteria to have substrate inhibition during the bacterial growth [79].

4. Conclusion

The growth of the bacterial community indicates slower growth rate behaviour and become growth-limiting at and above 1% (w/v) of NaCl, 2 g/L of nitrogen concentration, 1 g/L of yeast extract, 5% (v/v) of WCO concentration. The best-fitted model found was Teissier for the effects of NaCl, yeast extract and WCO at different concentrations on the bacterial growth rate with R^2 at 0.967, 0.989 and 0.830, respectively and different concentrations of nitrogen source showed that R^2 for the Aiba model was displayed at 0.959 with the lowest prediction error. The ranges of several parameters between the factors and model were generally in agreement among different models and differences were small. The maximum specific growth rate (μ_{max}), half-saturation coefficient (K_s) and substrate inhibitory constant (K_i) were obtained as these parameters are important in bioremediation application in a cold environment especially in the condition where the process of biodegradation depends on the population of the bacterial growth. Plus, the mathematical model is important in large-scale application of biodegradation, which allows selecting the optimal operating conditions.

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