Algae growth stabilization and prediction model matrix of soil sludge

[1]S. Siregar, [2]M.N.M. Pauzi, [3]C.M.M. Kasim, [4]S.H.M. Yusof, [5]K. Arumugam, [6]N. S. Yaacob, [7]M. Arifin

[1]Senior Lecturer, [2]Lecturer, [3]Lecturer, [4]Lecturer, [5]Student, [6]Senior Lecturer, [7]Assoc. Prof
Faculty of Engineering and Biotechnology,
Universiti Selangor, Bestari Jaya, Selangor, Malaysia

Abstract: **The pattern growth of the three algae species – PRK1-E01, C. Vulgaris and Nephrochlamys Subsolitaria which are found in the sludge within the Sabak Bernam region is not stable. The unstable distribution matrix of the algae growth is derived to determine a stable growth by modeling a transition matrix and stable distribution probability using Marcov Chain methodology. Thus, a stable algae growth can be predicted.**

Keywords: **Algae growth; Marcov Chain; Sludge extraction; Distribution matrix; stable probability**

I. Introduction

Sludge is a residuum that comprises adsorbed and inexplicable impurities which can be formed during a wastewater cure. It can also be defined as the fully nutrient rich organic materials. Those bio solids is recycled through soil application into a healthy benefits has been an exceedingly esteemed regular practice in numerous nation in the world by [1]. As an alternative, the recycling process in this study uses 25% to 89% of the bio solids as suggested by [2] and [3].

Other than that, mass culturing of high-value microalgae sorts is utilized from the aquaculture slush. [4], [5], [6] have established that microalgae development in aquaculture wastewater is conceivable. Moreover, it can be used in the construction of valuable produces such as the cosmeceuticals, nutraceuticals, aquaculture feeds, pharmaceuticals, and biofuels.

Microalgae is a collection of eukaryotic plants that are generally found in sea water and fresh water. This type of algae is able to withstand temperatures due to sunlight, control the level of water salinity, wide pH value, and light intensity either in water reservoirs or in the desert. Some important elements such as phosphorus, nitrogen, silica and iron as well as inorganic nutrients needed by microalgae. Micronutrients are also important for the reproduction of algae. Microalgae have carbon-rich compounds needed in pharmaceuticals, biofuels, animal foods, cosmetics, supplements, and many more uses (see [7] and [8]). [9] State that they have also produced a wide range of bio products such as bioactive compounds, antioxidants, vitamins, polysaccharides, pigments, proteins, and lipids.

In order to analyze and predict the future behavior, the Markov chain model is being utilized by many researchers in different application. The following references signify the applicability of Markov chain model in this context. Few of the examples, [18] utilizes Markov chain model to predict the possible states by illustrating the performance of the top two banks which are Guarantee Trust bank of Nigeria and First bank of Nigeria. They used six years data from 2005 to 2010. [19] implements a Markov chain model in forecasting the stock market trend in China. [20] introduce Markov chain model to forecast stock market trend of Safaricom share in Nairobi Securities Exchange in Kenya. Mettle, [21] uses Markov chain model with finite states to analyze the share price changes for five different randomly selected equities on the Ghana Stock Exchange and [22] consider forecasting model based on non-homogenous index sequence. But, none of them have utilized Marcov Chain methodology for the application of algae growth prediction and stability.

II. Methodology

Preparing of Sludge

Sludge was collected from aquaculture site in Sabak Bernam shrimp pond. One (1) kg of sample is collected from the site. Course particles in the sludge is removed and oven dries at 60°C until moisture is removed. After the drying process, dried sludge is grinded and sieved for omogenization.

Sludge Extraction

Aqueous extraction method is applied on the sludge models at an ambient temperature of 105°C. Extraction method carried out to the sludge sample was 105°C. Sludge mockup, 20 g of dried sludge was assorted with the 200 ml of ultra-pure water in Schott bottles. Then, sample was centrifuged using Beckmen Allegra X-30R centrifuge machine at 2500 rpm for 15 minutes. Supernatant was filtered using Whatman Glass Microfiber Filter (GF/F) 0.7µm and filtrates were kept in freezer for further analysis.

Preparation of Microalgae

Three microalgae species were used in this study such as *Chlorella vulgaris* (TRG 6), *Nephroclamys subsolitaria* (KDH 3 - C05) and unknown species (PRK 1 – E01). Microalgae sp. were composed from Peninsular Malaysia and, the genus, species of the microalgae recognized by UMT - University Malaysia Terengganu. *C. vulgaris* was a marine microalga while *N. subsolitaria* and PRK 1 – E01 were freshwater microalgae.

Subdivision of microalgae sp.

C. vulgaris uses Conway media and the other two microalgae use BBM as artificial growth medium. Three 50 ml conical flasks were autoclaved. 50 ml of Conway media or BBM was poured into the conical flasks and 1000 µl of microalgae was then added to the media. The sample was shake well and incubated at 25°C for further uses. After one or two weeks of incubation, the strains were used to test on sludge extracts. Subculture of all microalgae were conducted to avoid any cross-contamination in pure culture.

Microplate Incubation Technique

Microplate contains 96 wells which can be filled up to 200µl of solution as shown in **Fig. 1**.
175 µl of appropriate media, 5 µl of extracted sludge and 20 µl of algae were added in each well to record the exponential segment of microalgae. Control wells were also included in every test.

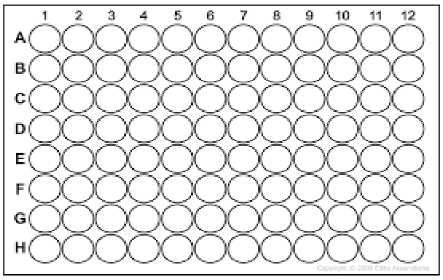

Figure 1. Microplate with 96 wells**.**

The ambient temperature at 25°C, source of light at 2,500 lux within 12-hour cycle period and 12-hour dark are few of the parameters. The microalgae growth is being monitored 24 hours to determine the optical density (OD) at 680 nm with the used of microplate reader Infinite M200 PRO (Tecan, Austria). The microalgae development is governed by evaluating the OD, by [23] as described it in the absorption of visible radiation in which the chlorophyll adsorption peak is approximately at the value of 680 nm. The OD of all the 96-wells are calculated for every 24 hours.

Discrete-Time Marcov Chains

Considers a system as one of a countable or finite state space where, $S \subseteq \{0,1,2,\ldots\}$.

Definition 2.1. By [24] *A discrete-time of stochastic process* $\{X_n : n \geq 0\}$ *with a state space* $S \subseteq \{0,1,2,\ldots\}$ *is called a discrete-time Markov chain (Mc) if and only if it has a Markov property (Mp),*

$$P\left(X_{n+1} = j \middle| X_n = i, X_{n-1} = i_{n-1}, \ldots, X_0 = i_0 \,|\right) = P\left(X_{n+1} = j \middle| X_n = i|\right). \quad (1)$$

for all $n \geq 0$, $i, j, i_0, i_1, \ldots, i_{n-1} \in S$ (the probability of the next state given the current state and the entire past depends only on the current state). The Mp as shown in Equation 1 is a constraint on the memory of the process: knowing the immediate past means the earlier outcomes are no longer relevant.

Transition Matrix

[25] states that if a Mc has *k* promising positions, which can be labelled as 1, 2, …, *k*. The probability of the arrangement is in the state *i* at any surveillance after it is in the state *j* at the previous surveillance as represented by p_{ij} and is called the transition possibility from state *j* to state *i*. The matrix $P = [p_{ij}]$ is known as the transition matrix. For example, in a three-state Mc, it has the form of a new state as shown in the Equation 2,

$$\begin{array}{cc} \begin{array}{ccc} 1 & 2 & 3 \end{array} & \\ \begin{bmatrix} p_{11} & p_{12} & p_{13} \\ p_{21} & p_{22} & p_{23} \\ p_{21} & p_{32} & p_{33} \end{bmatrix} & \begin{array}{c} 1 \\ 2 \\ 3 \end{array} \end{array} \tag{2}$$

$$p_{1j} + p_{2j} + \ldots + p_{kj} = 1 \tag{3}$$

in which, if the classification is in state j at one surveillance, it is assured to be in one of the k probable position at the next surveillance for the possibility models of the three (3) algae species - PRK1-E01, C. vulgaris (TRG 6) and Nephrochlamys subsolitaria.

Algae growth data

The daily Alga Growth Data of PRK1-E01, C. vulgaris (TRG 6) and Nephrochlamys subsolitaria during Day 2, Day 3 and Day 4 are gathered in Table 1 and Fig. 2.

Table 1. The Alga growth data of three species from Sabak Bernam Sludge within 3-day period.

ALGAE SPECIES	DAY 2	DAY 3	DAY 4
PRK1-E01	0.07	0.09	0.11
C. vulgaris (TRG 6)	0.06	0.12	0.17
Nephrochlamys subsolitaria	0.05	0.10	0.18

Figure 2. The algae growth data for 4-day period.

Algae growth

The algae growth data is selected at the highest value of each day with the three different species at different day ' growth trends of PRK1-E01, *C. Vulgaris* (TRG 6) and Neophrochlamys Subsolitaria during day 2,3 and 4 in (10^{-2})

Algae Species	DAY 2	DAY 3	DAY 4	Total
PRK1-E01	7	9	11	27
C. vulgaris (TRG 6)	6	12	17	35
Nephrochlamys subsolitaria	5	10	18	33
Total	**18**	**31**	**46**	**95**

III. Results and Discussion

Unstable Distribution Growth

The algae growth is distributed equally which is being produced randomly means that each type of algae has equal access information. The probabilities of the Mp are estimated through technical analysis in which PRK1-EO1 is growth at 26% followed by the next day in which C. Vulgaris (TRG 6) at 15% and Nephrochlamys subsolitaria at 55% and so on.

The probability of each algae growth can be determined by the ratio as stated in Equation 4 and the results are captured in Table 3 in which the transition schematic diagram is demonstrated in Figure 3.

$$P(Algae) = \frac{Algae\ growth}{Total\ \ Algae\ growth} \tag{4}$$

Table 3. The probability growth from day 2,3 and 4

To / From	Day 2	Day 3	Day 4
	PRK1-E01	C. vulgaris (TRG 6)	Nephrochlamys subsolitaria
PRK1-E01	0.26	0.33	0.41
C. vulgaris (TRG 6)	0.17	0.34	0.49
Nephrochlamys subsolitaria	0.15	0.30	0.55

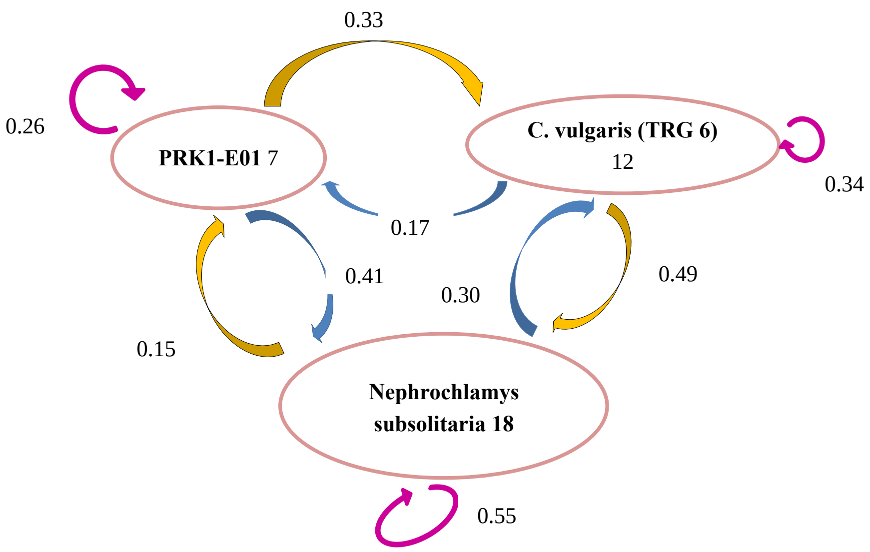

Figure 3. Transition diagram

Computation Growth

The computation process to predict the algae growth trends for unstable distribution within day 2 to 4 is captured in Table 4.

Table 4. Probability Table of Markov Chains

Algae Species	Day 2	Day 3	Day 4	Total
PRK1-E01	0.26	0.33	0.41	1.0
C. vulgaris (TRG 6)	0.17	0.34	0.49	1.0
Nephrochlamys subsolitaria	0.15	0.30	0.55	1.0
Total	0.58	0.97	1.45	
	$\neq 1$	$\neq 1$	$\neq 1$	

Derivation from unstable to stable distribution – **Table 4** shows that the total probability of the day 2, 3 and 4 are not equal to 1, then the distribution is unstable. Therefore, to derive the unstable into a stable distribution, the algae growth probability has to be derived first as follows,

Lets,

Tot = Total number of PRK1-E01, C. vulgaris (TRG 6) and Nephrochlamys subsolitaria in Table 2 (yellow highlight), resulting a total of 37.

a_{11} = PRK1-E01

a_{22} = C. vulgaris (TRG 6)

a_{33} = Nephrochlamys subsolitaria

Equation (4) can be derived into Equation (5) as follows,

$$P(a_{nn}) = \frac{Algae\ growth}{\sum_{n=1}^{3} a_{nn}} \tag{5}$$

From the above equation, thus the unstable distribution probability for PRK1-E01 is 0.19; unstable distribution probability for C. vulgaris (TRG 6) is 0.32; and the unstable distribution probability for Nephrochlamys Subsolitaria is 0.49.

Lets X_0 represents the row matrix of the unstable algae growth probability with the three species which is represented by a vector with Algae growth Space as S= {PRK1-E01, C. vulgaris (TRG 6), Nephrochlamys subsolitaria}.

Thus,

$$X_0 = (P(a_{11}) \quad P(a_{22}) \quad P(a_{33})) \tag{6}$$

or,

$$X_0 = (0.19 \quad 0.32 \quad 0.49)$$

Then, the probability of Table 4 can be transformed into the transition matrix as shown in Equation (7),

$$A = \begin{array}{c} \text{To} \\ \begin{bmatrix} 0.26 & 0.33 & 0.41 \\ 0.17 & 0.34 & 0.49 \\ 0.15 & 0.30 & 0.55 \end{bmatrix} \end{array} \text{From} \tag{7}$$

The probability matrix has the ability to predict what is going to happen in the future based apart from what is happening currently and how things going to be changed.

Determination of future algae growth

The future algae growth is the matrix of the current algae growth transition probabilities [26]. Thus,

$$X_{n+1} = X_n A, \quad n = 0,1,2,... \tag{8}$$

where, X_{n+1} = Next Algae Growth,

A = Probability Matrix

X_n = Initial Algae Growth

Lets. X_n = *(PRK1-E01, C.vulgaris (TRG 6), Nephrochlamys subsolitaria),*
when $n = 0 \rightarrow$ One day after Day 2 : Day 3
Thus,

$$X_1 = X_0 A \tag{9}$$

or,

$$X_1 = \begin{pmatrix} 0.19 & 0.32 & 0.49 \end{pmatrix} \begin{pmatrix} 0.26 & 0.33 & 0.41 \\ 0.17 & 0.34 & 0.49 \\ 0.15 & 0.30 & 0.55 \end{pmatrix}$$

or,

$$X_1 = \begin{pmatrix} \underset{\text{PRK } X_2}{0.1773} & \underset{\text{Vulgaris } X_2}{0.3185} & \underset{\text{Neph } X_2}{0.5042} \end{pmatrix}$$

Therefore, the total up of the probabilities above is equal to 1.

Determination of algae growth distribution

The algae growth is determined by multiplying each algae probability with the total number of the three algae PRK1-E01, C.Vulgaris (TRG 6) and Nephrochlamys subsolitaria.

Therefore, each algae growth for PRK1-E01, C. Vulgaris (TRG 6) and Nephrochlamys subsolitaria are determined as 6.5601, 11.784 and 18.655 respectively.

So, the matrix of algae growth is denoted by, $X_1 = (6.5601 \ 11.7845 \ \ 18.6554)$

When $n = 1 \rightarrow$ Two days after day 2 : day 4

$$X_2 = X_1 A$$

$$= \begin{pmatrix} 0.1773 & 0.3185 & 0.5042 \end{pmatrix} \begin{pmatrix} 0.26 & 0.33 & 0.41 \\ 0.17 & 0.34 & 0.49 \\ 0.15 & 0.30 & 0.55 \end{pmatrix}$$

$$X_2 = \begin{pmatrix} 0.175873 & 0.318059 & 0.506068 \end{pmatrix}$$

$$= \begin{pmatrix} \underset{\text{PRK } X_2}{0.175873} & \underset{\text{Vulgaris } X_2}{0.318059} & \underset{\text{Neph } X_2}{0.506068} \end{pmatrix}$$

Thus, the total up of the probabilities of the above is equal to 1.

Then again, the distribution of the algae growth is determined for the second attempt by multiplying the above first round probability by the total number of the three algae PRK1-E01, C. Vulgaris (TRG 6) and Nephrochlamys subsolitaria,

Therefore, each algae growth for PRK1-E01, C. Vulgaris (TRG 6) and Nephrochlamys subsolitaria are determined in the second attempt as 6.507301, 11.768183 and 18.724516 respectively.

So, the matrix of Algae Growth is denoted by,

$$X_2 = \begin{pmatrix} 6.507301 \; 11.768183 \; 18.724516 \end{pmatrix}$$

So, the diagonalize process determines the second attempt for the algae growth matrix is as follows,

$$Let\ A = \begin{pmatrix} 0.26 & 0.33 & 0.41 \\ 0.17 & 0.34 & 0.49 \\ 0.15 & 0.30 & 0.55 \end{pmatrix}$$

Thus, the eigenvalues of A are $\lambda_1 = 1,$ $\lambda_2 = 0.1177,$ and $\lambda_3 = 0.0323.$

To determine the eigenvectors corresponding to the eigenvalue $\lambda = 1,$ compute the null space of $A - 1.000I.$

Therefore $E_1 = \begin{pmatrix} x \\ y \\ z \end{pmatrix} = \begin{pmatrix} 1 \\ 1 \\ 1 \end{pmatrix}$, $E_{0.1177} = \begin{pmatrix} x \\ y \\ z \end{pmatrix} = \begin{pmatrix} 0.9448 \\ -0.0002 \\ -0.3277 \end{pmatrix}$ and $E_{0.0323} = \begin{pmatrix} x \\ y \\ z \end{pmatrix} = \begin{pmatrix} 0.6270 \\ -0.7390 \\ 0.2466 \end{pmatrix}$

So, the corresponding eigenvectors are

$$v_1 = \begin{pmatrix} 1 \\ 1 \\ 1 \end{pmatrix} \quad v_2 = \begin{pmatrix} 0.9448 \\ -0.0002 \\ -0.3277 \end{pmatrix} \quad \text{and} \quad v_3 = \begin{pmatrix} 0.6270 \\ -0.7390 \\ 0.2466 \end{pmatrix}$$

Next to verify that $D = P^{-1}AP,$ where

$$P = \begin{pmatrix} 1 & 0.9448 & 0.6270 \\ 1 & -0.0002 & -0.7390 \\ 1 & -0.3277 & 0.2466 \end{pmatrix} \quad \text{and} \quad P^{-1} = \begin{pmatrix} 0.1757 & 0.7148 & 0.2375 \\ 0.3180 & 0.2759 & -0.9229 \\ 0.5063 & -0.9907 & 0.6854 \end{pmatrix}$$

So that $D = P^{-1}AP = \begin{pmatrix} 1 & 0 & 0 \\ 0 & 0.1177 & 0 \\ 0 & 0 & 0.0323 \end{pmatrix}$

or, $D^n = \begin{pmatrix} 1^n & 0 & 0 \\ 0 & 0.1177^n & 0 \\ 0 & 0 & 0.0323^n \end{pmatrix}$

While $A^n = PD^nP^{-1}$

$$= \begin{pmatrix} 1 & 0.9448 & 0.6270 \\ 1 & -0.0002 & -0.7390 \\ 1 & -0.3277 & 0.2466 \end{pmatrix} \begin{pmatrix} 1^n & 0 & 0 \\ 0 & 0.1177^n & 0 \\ 0 & 0 & 0.0323^n \end{pmatrix} \begin{pmatrix} 0.1757 & 0.7148 & 0.2375 \\ 0.3180 & 0.2759 & -0.9229 \\ 0.5063 & -0.9907 & 0.6854 \end{pmatrix}$$

$$= \begin{pmatrix} 1^n & 0.94480(0.1177^n) & 0.6270(0.0323^n) \\ 1^n & -0.0002(0.1177^n) & -0.7390(0.0323^n) \\ 1^n & -0.3277(0.1177^n) & 0.2466(0.0323^n) \end{pmatrix} \begin{pmatrix} 0.1757 & 0.7148 & 0.2375 \\ 0.3180 & 0.2759 & -0.9229 \\ 0.5063 & -0.9907 & 0.6854 \end{pmatrix}$$

$$= \begin{pmatrix} 0.1757+0.3(0.1177^n)+0.32(0.0323^n) & 0.7148+0.26(0.1177^n)-0.62(0.0323^n) & 0.2375+0.3(0.1177^n)+0.32(0.0323^n) \\ 0.1757-0.64(0.1177^n)-0.37(0.0323^n) & 0.7148-0.00006(0.1177^n)+0.73(0.0323^n) & 0.2375+0.0002(0.1177^n)-0.51(0.0323^n) \\ 0.1757-0.10(0.1177^n)+0.12(0.0323^n) & 0.7148-0.09(0.1177^n)-0.24(0.0323^n) & 0.2375+0.3(0.1177^n)+0.17(0.0323^n) \end{pmatrix} \tag{10}$$

Since $X_{n+1} = AX_n$ in Equation (10).

Then,

$$X_n = X_0A^n \tag{11}$$

where $n = 0,1,2,...$ and A^n is denoted by Equation (11).

If the future growth is becoming the same with the initial growth, then the chain shall be stopped means that the distribution becomes stable.

Then, the values of X_n where $(n = 0,1,2,...7)$ can be summarized in the table form as shown in Table 5 as follows,

Table 5 demonstrates that the distribution is stabled at seventh (7^{th}) attempt by multiplying the probability and algae growth value of $n = 7$. The distribution value for the next state is the same as before. Then, the algae growth matrix begins to be converged. Therefore, the solution is concluded that the probabilities are converged to a steady state algae growth, thus n → 7 means that the values of 6.500351527 for PRK1-E01, 11.76563604 for C. vulgaris (TRG 6) and 18.73401243 for Nephrochlamys subsolitaria are finalized.

This solution concludes that the probabilities is converged to a steady algae growth, thus, n → 7 means that the values of 6.500351527 for PRK1-E01, 11.76563604 for C. vulgaris (TRG 6) and 18.73401243 for Nephrochlamys subsolitaria are finalized.

If the same result is obtained with the unstable by the row unstable (transpose method), then the future growth is becoming the same with the initial growth, so the Mc should be on hold. This means that the distribution is stable.

Table 4. The summary of the distribution probability values for X_n

N	X_n	*(PRK1-E01; C.Vulgaris(TRG6);Nephrochlamys S)*	**Corresponding Eigenvectors**
0	X_0	(0.19; 0.32; 0.49)	0
1	X_1	(0.1773; 0.3185; 0.5042)	E_1=(0.01267; 0.0015; -0.0142)
2	X_2	(0.175873; 0.318059; 0.506068)	E_2=(1.427(10_{-3}); 4.41(10^{-4}); -0.001868)
3	X_3	(0.17570721; 0.31799855; 0.50629424)	E_3=(1.6579(10^{-4}); 6.045(10^{-5}); -2.2624(10^{-4}))
4	X_4	(0.175685477641; 0.3179911583; 0.5063210776)	E_4=(1.94459(10^{-5}); 7.3917(10^{-6}); -2.68376(10^{-4}))
5	X_5	(0.175685477217; 0.317990279255; 0.506324243528)	E_5=(4.24(10^{-8}); 8.79(10^{-6}); -3.166(10^{-6}))
6	X_6	(0.175685208078970; 0.317990175486710; 0.506324616434320)	E_6=(2.69(10^{-7}); 1.037(10^{-7}); -3.73(10^{-7}))
7	X_7	(0.175685176398421; 0.317990163261837; 0.506324660339742)	$E_7 \rightarrow 0$ the system stable
8	X_8	(0.175685172669063, 0.317990161822426; 0.506324665508511)	$E_8 \rightarrow 0$ the system stable

Figure 4 shows the new transition diagram in which it is designed after the distribution is stable. Then, the probability matrix and initial algae growth is utilized to calculate the next algae growth. If the attemption goes on the next algae growth continuously, then the algae growth is kept changing very quickly and the changes can be seen either lower or higher. Then, it drives the algae growth matrix begins to converge to certain particular values.

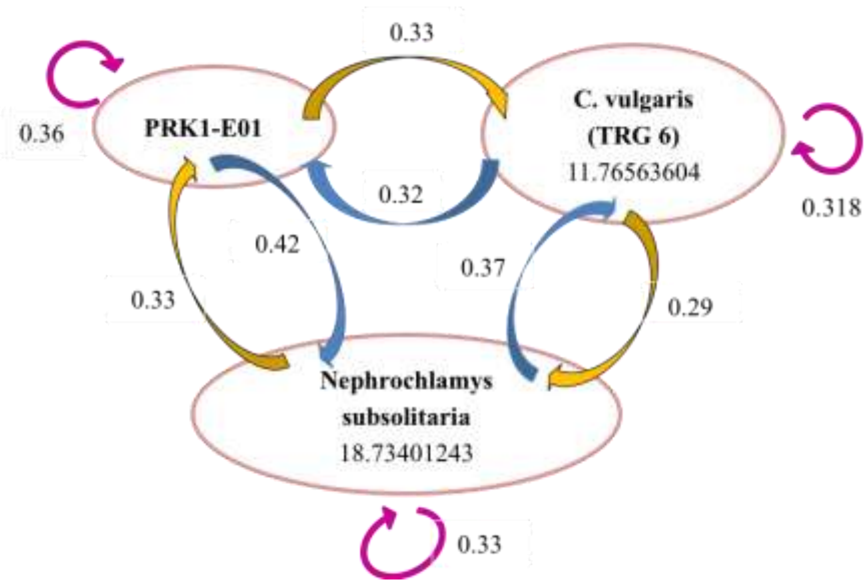

Figure 4. The Result for New Stability Transition Growth Diagram

The n^{th} value depends on the situation of getting the stability of the distribution matrix. The stable distribution matrix demonstrates that a new algae growth value shall be obtained on the following day. In the analysis above that the distribution is stabled at the 8^{th} attempt by multiplying the probability with the algae growth value of $n = 7.$ Then, the distribution will be valued for the next attempted state as exactly the same as the earlier attemption one step behind . Now, the algae growth matrix begins to be converged.

IV. Conclusion and Recommendation

This attemption steps or systems methodology can be used as an example before any new products are introduced into the market. As example a new product which are not yet available in the market. In order for a new product to be penetrated into the marketplace, a marketing and commercial study shall be done at all optimum levels of those study and evaluation.

An attempted curiosity attitude from customers is a norm at the initial state to purchase any new products in the marketplace as compared to their favorite products in which they are used to purchase. If the new product is good or matched the customer's needs, the they will switch to the new brand for the second attempt. If it is bad product, the customer will not continue to purchase it rather maintaining the previous brand. Therefore, the probability matrix using Markov Chains method is very useful to predict on how a new product shall be able to penetrate into the current marketplace competing with any established brands.

V. Acknowledgment

This Research work was supported by JST, JICA, SATREPS through the project for COSMOS; Grant No. JPMJSA1509, and the SATREPS-COSMOS Matching Fund from the Ministry of Higher Education Malaysia – MOHE.

VI. Conflict of Interest

All authors declare no conflicts of interest in this paper.

References

[1] S. T. Madariaga and S. L. Marín, Sanitary and environmental conditions of aquaculture sludge. *Aquaculture research,* **48(4)** (2017), 1744-1750. https://doi.org/10.1111/are.13011

[2] C. Bonnin, Organic pollutants and sludge – the French experience in: Research the Sludge Directive, A conference on Sewage Sludge, Brussels, (2001), Retrieved 30 Oct 2018, from htpp://europe.eu.int/comm/environments/ sludge/ conference.htm.

[3] Z. Guo, Y. Liu, H. Guo, et. al. Microalgae cultivation using an aquaculture wastewater as growth medium for biomass and biofuel production. *J. Environmental Sciences,* **25 (**2013), S85-S88. https://doi.org/10.1016/S1001-0742(14)60632-X

[4] Chloe Kemigabo Lazaro W. Jere Daniel Sikawa Charles Masembe Jeremiah Kang'ombe Mohsen Abdel‐Tawwab, "Growth response of African catfish, Clarias gariepinus (B.), larvae and fingerlings fed protease‐incorporated diets", 14 February 2019 https://doi.org/10.1111/jai.13877

[5] M. H. Michels, M. Vaskoska and M.H. Vermuë, et. al., Growth of Tetraselmis suecica in a tubular photobioreactor on wastewater from a fish farm. *Water research,* **65** (2014), 290-296. https://doi.org/10.1016/j.watres.2014.07.017

[6] P. Das, S. S. Aziz and J. P. Obbard, Two phase microalgae growth in the open system for enhanced lipid productivity. *Renewable Energy,* **36(9)** (2011), 2524-2528. https://doi.org/10.1016/j.renene.2011.02.002

[7] L. Barsanti, P. Coltelli, V. Evangelista, A.M. Frassanito, V. Passarelli, N. Vesentini and P. Gualtieri, Oddities and curiosities in the algal world. In: *Algae toxins: nature, occurrence, effect and detection*. Dordrecht: Springer, (2008), 353–391.

[8] L. Brennan and P. Owende, Biofuels from microalgae a review of technologies for production, processing and extractions of biofuels and co-products. *Renewable and sustainable energy reviews,* **14(2)** (2010), 557-577. https://doi.org/10.1016/j.rser.2009.10.009

[9] M. Huesemann, B. Crowe and P. Waller, et. al.. A validated model to predict microalgae growth in outdoor pond cultures subjected to fluctuating light intensities and water temperatures. *Algal Research,* **13** (2016), 195-206. https://doi.org/10.1016/j.algal.2015.11.008

[10] Q. Zhao, A. N. Chen, S.X. Hu, et. al., Microalgal Microscale Model for Microalgal Growth Inhibition Evaluation of Marine Natural Products. *Scientific reports, 8*(1), (2018), 10541. https://doi.org/10.1038/s41598-018-28980-z

[11] J. Van Wagenen, S. L. Holdt and D. De Francisci, et. al., Microplate-based method for high-throughput screening of microalgae growth potential. *Bioresource technology,* **169** (2014), 566-572. https://doi.org/10.1016/j.biortech.2014.06.096

[12] A. Pacheco, I. Hernández□Mireles and C. García□Martínez, et. al., Microplates as a microreactor platform for microalgae research, *Biotechnology progress*, ***29*(3)** (2013), 638-644, https://doi.org/10.1002/btpr.1721.

[13] C. Blaise and J. F. Férard. (2005), Overview of contemporary toxicity testing. In Small-Scale Freshwater, *Toxicity Investigations*, (2013), 1-68, Springer, Dordrecht, https://doi.org/10.1007/1-4020-3120-3.

[14] D. St□Laurent, C. Blaise and P. MacQuarrie, et. al., Comparative assessment of herbicide phytotoxicity to *Selenastrum capricornutum* using microplate and flask bioassay procedures. *Environmental Toxicology and Water Quality,* **7(1)** (1992), 35-48. https://doi.org/10.1002/tox.2530070104

[15] R. Rojíčková, D. Dvořáková and B. Maršálek, The use of miniaturized algal bioassays in comparison to the standard flask assay. *Environmental Toxicology and Water Quality: An International Journal,* ***13*(3)** (1998), 235-241. https://doi.org/10.1002/(SICI)1098-2256(1998)13:3%3C235::AID-TOX5%3E3.0.CO;2-8

[16] D. N. Choji, S. N. Eduno & G. T. Kassem, Markov Chain Model Application on Share Price Movement in Stock Market: *Journal of Computer Engineering and Intelligent Systems. www. iiste.org,* **4(10)** (2013), 84-95.

[17] D. Zhang, X. Zhang, Study on Forecasting the Stock Market Trend Based on Stochastic Analysis Method: *International Journal of Business and Management.* **4(6)** (2009), 163-170.

[18] S. Otieno, E. O. Otumba and R. N. Nyabwanga, Application of Markov Chain to Model and Forecast Stock Market Trend: A Study of Safaricom Shares in Nairobi Securities Exchange, Kenya: *International Journal of Current Research,* (2015), 14712-14721.

[19] F. O. Mettle, Quaye ENii Boi and R. A. Laryea, A Methodology for Stochastic Analysis of Share Price of Markov Chains with Finite States, *SpringerPlus,* **3(1)** (2014), 657, doi: 10.1186/2193-1801-3-657

[20] N. M. Xie, S. F. Liu, Y. J. Yang and C. Q. Yuan, On Novel Grey Forecasting Model Based on Non-Homogenous index Sequence. *Applied Mathematical Modelling,* 37, (2013), 5059-5068. https://dx.doi.org/10.1016/j.apm.2012.10.037

[21] K. Dziosa and M. Makowska, Monitoring of Chlorella sp. growth based on the optical density measurement. *Problemy Eksploatacji*. (2016)

[22] Damjan Škulj, Discrete time Markov chains with interval probabilities. *International Journal of Approximate Reasoning*, **50**(2009), 1314–1329.

[23] H. Anton and C. Rorres, *Elementary Linear Algebra,* (8th ed.). Canada: Techsetters, Inc, 2000.

[24] D. Poole, *Linear Algebra: A Modern Introduction,* United States of America: Robert W. Pirtle, 2011.