TREATMENT OF SUSPENDED SOLIDS IN SEDIMENT TRAP USING BIOFLOCCULANT PRODUCED BY *Bacillus sphaericus* UPMB10

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INTRODUCTION

River water quality in Malaysia has been deteriorating throughout the years. Population booms and development as a whole are major causes of this problem as pollution inputs increases parallel with the increment of human activities. One of the major pollutants that were identified as the cause of river water pollution in Malaysia is suspended solids (SS) pollution (Malaysia Environmental Quality Report, 2006, 2007).

Suspended solids pollution occurs when sediments from surface runoff are flushed-down into the river. Nowadays, this natural occurring process became a rising concern as excessive amount of sediments are entering the river system due to the intensive land clearing activity done for development purposes. Deforestation of land will leave the land bare; exposing it to heavy rains and thus will lead to erosion problems.

As a requirement for any development projects, every land clearing activity should be equipped with a sediment trap that allows the surface runoff to be collected first before entering the river system. This is where the sediments carried with the runoff are allowed to settle as to reduce the inputs of suspended solids into the river water. However, over development occurred have deteriorated the natural flow of this event, thus, even the sediment traps constructed might not be enough to solve the problem.

Therefore, this research aims to develop a solution by using bioflocculant produced by bacteria to enhance flocculation and settlement of suspended particles in sediment traps before the resulting runoff being discharge into the river water system.

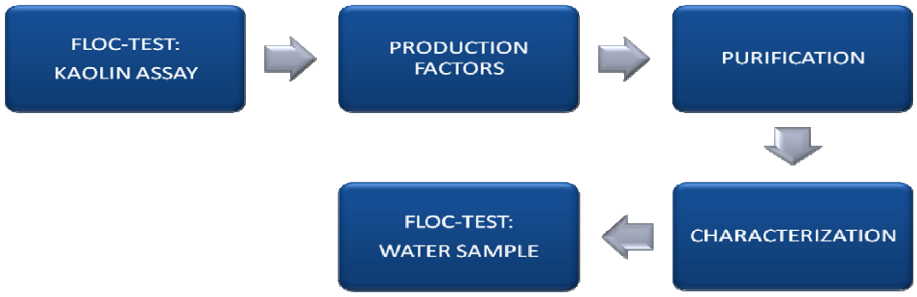
OBJECTIVES

1. To extract a potential bioflocculant produced by *Bacillus sphaericus* UPMB10 for the treatment of suspended solids

2. To evaluate the flocculating capabilities of the bioflocculant

3. To simulate the treatment process of suspended solids in sediment trap by using the bioflocculant

RESEARCH METHODOLOGY



Kaolin Assay

1. 5g of kaolin clay (supplied by Kaolin (M) Sdn. Bhd) with an average size of 4-5µm is suspended in 1L ultra pure water. pH of the suspension is adjusted to 6.8 with HCl and NaOH.

2. 45ml of the suspension is inserted into 100ml conical flasks and is autoclaved at 121oC for 15min. The flasks are then left to cool at room temperature.

3. Four treatments are subjected to the suspensions which are:

CONTROL = 45ml kaolin + 4.5ml SUPW + 0.5 sterile TSB
(+) CATION = 45ml kaolin + 4.5 CaCl2 (0.1%) + 0.5 sterile TSB
(+) BACTERIA = 45ml kaolin + 4.5ml SUPW + 0.5 UPMB10
(+) BACTERIA (+) CATION = 45ml kaolin + 4.5ml CaCl2 (0.1%) + 0.5 UPMB10

The treatments are done in three replicates.

4. All flasks with respective treatments are transferred onto an orbital shaker and are agitate at the speed of 200rpm for 30s and are left to settle for 5min.

5. The measurements of absorbency (optical density) of the upper phase of the suspensions are measured using a spectrophotometer at the wavelength of 550nm. The flocculation activity is determined by the equation below:

$$\text{Flocculating activity (\%)} = \frac{A - B}{A} \times 100$$

Where;
A = OD of control at 550 nm
B = OD of sample at 550 nm

6. Growth of UPMB10 is determined by the measurement of optical density at 660nm.

7. The pH and temperature of the resulting suspension (after the flocculation test) was measured using a pH meter.

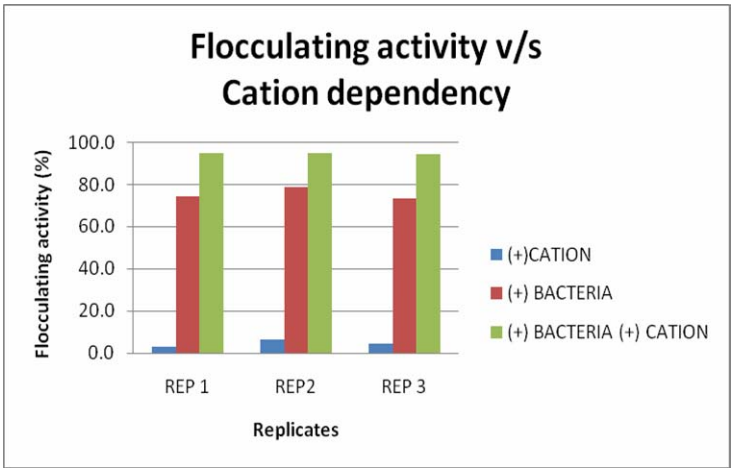
Production factors

1. Growth Stage
2. pH

RESULTS & DISCUSSION

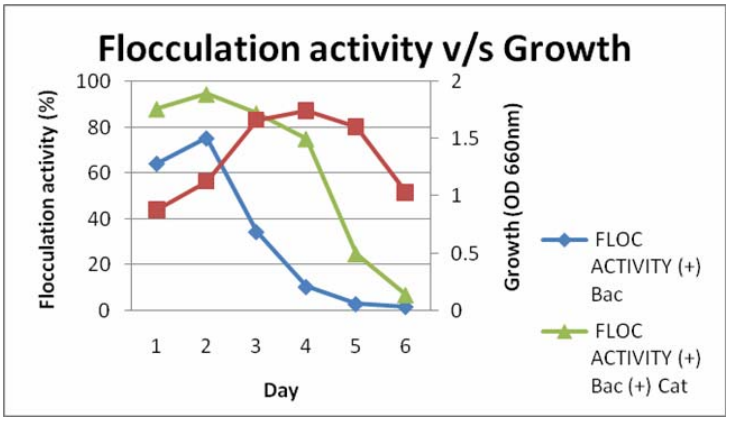
Flocculating activity (%) v/s Cation dependency

TREATMENTS	FLOCCULATING ACTIVITY (%)		
	REP 1	REP 2	REP 3
(+)CATION	3.0	6.4	4.6
(+) BACTERIA	74.1	78.5	75.3
(+) BACTERIA (+) CATION	94.6	94.7	94.6



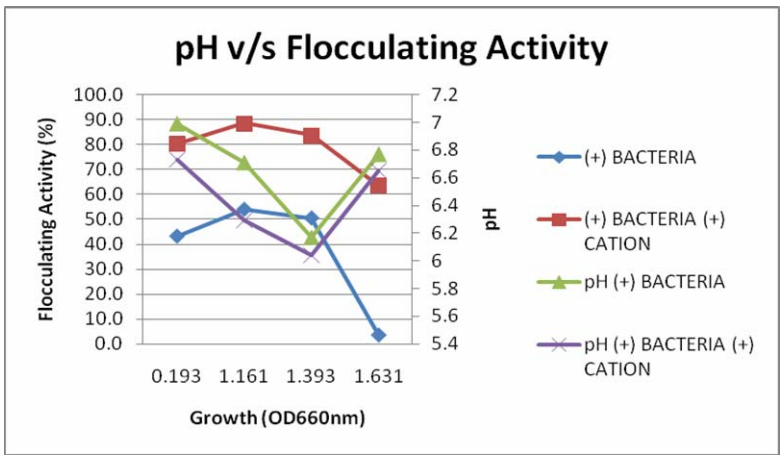
Flocculating activity (%) v/s Growth

Day	OD660	FLOC ACTIVITY (+) Bacteria (%)	FLOC ACTIVITY (+) Bac (+) Cat (%)
1	0.877	64.2	88.1
2	1.126	75.3	94.6
3	1.662	34.3	86.2
4	1.744	10.2	75.1
5	1.602	2.7	24.8
6	1.028	1.5	6.9



Flocculating activity (%) v/s pH (*note: using CaCl2 at 0.1% concentration)*

DAY	OD660	TREATMENT	pH	OD550	FLOC XTVT (%)
1	0.193	CONTROL	6.97	1.942	
		(+)CATION	6.55	1.705	12.2
		(+) BACTERIA	6.99	1.103	43.2
		(+) BACTERIA (+) CATION	6.73	0.381	80.4
2	1.161	CONTROL	6.97	1.982	
		(+)CATION	6.55	1.929	2.7
		(+) BACTERIA	6.71	0.912	54.0
		(+) BACTERIA (+) CATION	6.29	0.227	88.5
3	1.393	CONTROL	6.65	1.745	
		(+)CATION	5.75	1.722	1.3
		(+) BACTERIA	6.17	0.864	50.5
		(+) BACTERIA (+) CATION	6.40	0.286	83.6
4	1.631	CONTROL	6.89	1.775	
		(+)CATION	6.07	1.751	1.4
		(+) BACTERIA	6.77	1.712	3.5
		(+) BACTERIA (+) CATION	6.65	0.647	63.5



Flocculation assay using kaolin suspension showed that *Bacillus sphaericus* UPMB10 has potential flocculating ability with the highest flocculating activity achieved was 78.5%. The UPMB10 was found to produce a cation-dependent bioflocculant, as an increase of about 20% can be seen, giving the highest flocculating activity achieved with the addition of $CaCl_2$ was 94.7%. It is observed that the flocculating activity reaches it optimum at the early growth stage of UPMB10, at about 48hrs of incubation and starts to decline at about the 72hrs of incubation. This proves that the bioflocculant production occurs parallel to the bacterial growth at the early logarithmic growth stage. The decrease in flocculating activity is hypothesized to be caused by the lack of nutrient supplied by the broth, whereby the bioflocculant produced was being uptake by the bacteria as substitute for nutrients. This is because, bioflocculants are extra-cellular polymers excreted by the bacteria and it can be the form of proteins, polysaccharides or glycoproteins, which in turn are substrates of

nutrients for their growth. Further study on the effect of continuous supply of nutrients on the flocculating activity is still under study.

Effect of pH (separate experiment) were done do determine whether pH variations during growth might be the cause to the decrease of flocculating activity. The initial pH for the kaolin suspension used for every test is adjusted to pH6.8 as this is the optimum pH for UPMB10 growth. From the results, it can be said that bioflocculant favors pH of about 6.7 to 6.9 where the flocculating activity was found to be above 80%. For pH below pH6, the flocculating activity achieved was found to be only up to 50%. This results supports the fact that UPMB10 favors pH of around 6.8.

SIGNIFICANCE OF FINDING

The results showed that locally cultured bacterial strain *Bacillus sphaericus* UPMB10 can produce a potential bioflocculant with flocculating activity up to 94%. Tests done allowed a possibility of getting the optimum conditions for maximum bioflocculant production to be extract and purified for further study on its applicability on river water samples in a simulated sediment trap.