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# The Ability of Froth Formed without Chemicals to Hold Bacteria

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**Abstract.** Froth flotation is a solid-liquid separation technique that uses hydrophobicity as a driving force. Bacteria and other drinking water microorganisms tend to be hydrophobic and can be removed from water using this application. The biggest limitation against using froth flotation in the drinking water industry is the difficulty of producing froth without chemical "frothers" and holding bacteria in this froth without chemical collectors which deteriorate water taste and odor. Recently, researchers at the University of Sheffield described a method for producing froth using only water and compressed air. This has enabled froth flotation to be studied as an alternative to biocides for the removal of bacteria from drinking water.

This work examines the ability of froth, produced by controlling air pumping through a water column, to hold bacteria. Bacteria are moved to the top of the column and collected in the froth. The operating conditions determine the percentage of bacteria removed.

At optimum conditions, froth can hold up to $2\times10^8$ cfu/ml of bacteria. It has been found that air pumping at 130 l/min in a 20 cm diameter column will give the highest froth bacterial content. Time to reach stable froth bacterial concentration is decreased by increasing other variables.

## 1 Introduction

Until now, froth flotation techniques have been little used in the drinking water industry even though it is effective as a solid-liquid separation method that can be used to separate microorganisms from water. It has been avoided because chemical frothers and collectors have to be used which deteriorate water taste, odor and safety as the majority of them are alcohols and polyglycols (Finch and Zhang, 2014). To overcome this limitation, a system has been developed to produce froth using compressed air and water only (Hassan, 2015).

Separation of bacterial strains from water by froth flotation has been used for over sixty years (Boyles and Lincoln, 1958;Rubin et al., 1966;Bahr and Schugerl, 1992;Rios and Franca, 1997;Kulkarni, 2016). This technique has also been used to collect hydrophilic or less hydrophobic materials by attaching them to more hydrophobic one, for example the attachment of hydrophobic metal particles to bacteria to give them hydrophobic behavior and subsequent flotation (Smith et al., 1993;Nagaoka et al., 1999;Olivera et al., 2017). Algae have been separated (harvested) from suspensions using hydrophobicity in bubble columns (Levin et al., 1962). A recent trail for separating algae using micro-bubble flotation has given good results (Hanotu et al., 2012).

In an introduction to their work, Hanotu et al. (2012) state that separation efficiency is inversely proportional to bubble size because surface area is increased as bubble size decreases, increasing the probability of bubble-microorganism contact. Oppositely, as bubble size increases, buoyancy force and rising speed reducing bubble particle attachment changes (Hanotu et al., 2012). Therefore, the net effect of larger bubble size stills a gap that represents a good area to investigate.

Previously, it was believed that the optimum particle size for froth flotation is in the range of 88-500 microns (Zech et al., 2012). However, the recent work by Hanotu et al. (2012) and by Lertrojanachusit (2013) on carbon nano tubes indicates much smaller particles can be removed by such techniques.

With all these reasons and motivations, a trial should be made to investigate the ability of froth produced without chemicals to hold bacteria without using any chemical frothers and collectors.

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## 2 Materials and methods

### 2.1 Froth flotation column

A compact froth flotation system was designed using a transparent Perspex (Poly methyl methacrylate) tube 20 cm diameter and 2 meters length. A ceramic sparger 19 cm diameter with 50 micron pore size is fixed 30 cm above column base. The sparger base is joined to a 15 mm diameter tube that is connected to a compressor via a rotameter of (10-900) l/min. Five side streams are attached to the column at 30 cm intervals above the sparger i.e. 30, 60, 90, 120 and 150 cm above the sparger. These side streams are used to collect froth samples. A 200 liter tank is installed beside the system to collect distilled water from a still and provide a reservoir to the column. The capacity of column is approximately 60 liters when totally filled. The assembly is fixed on a steel rig (Figure 1).

### 2.2 Bacterial nutrient broth

Nutrient broth was prepared by mixing 15 g of nutrient broth (SIGMA-ALDRICH) in 1 liter of distilled water. When dissolved completely, it was autoclaved for sterilization. The broth was then inoculated with bacteria and incubated for 24 hours and 37 C°. The bacteria used in this work were the K-12 strain *Escherichia Coli* (Texas Red). The mother bacteria were kept deep frozen and subcultured as required.

### 2.3 Turbidity measurement

A turbidity meter (TurbiCheck, from Lovibond water testing Co.) was used to measure turbidity as a function of total bacterial content. This device is equipped with four calibration standards, 800, 200, 20 and less than 0.1 NTU. Calibration of this meter was carried out daily before use.

A standardization test was carried out to check the meter readings and range. One liter of inoculated nutrient broth was prepared according to (2.2) and measured for turbidity (NTU). Dilutions in distilled water were prepared to obtain the following dilutions: 1/2, 1/4, 1/8, 1/16…etc. The turbidity of these dilutions was measured also for NTU. The obtained relationship, which shows a direct proportionality, is given in (Figure 2).

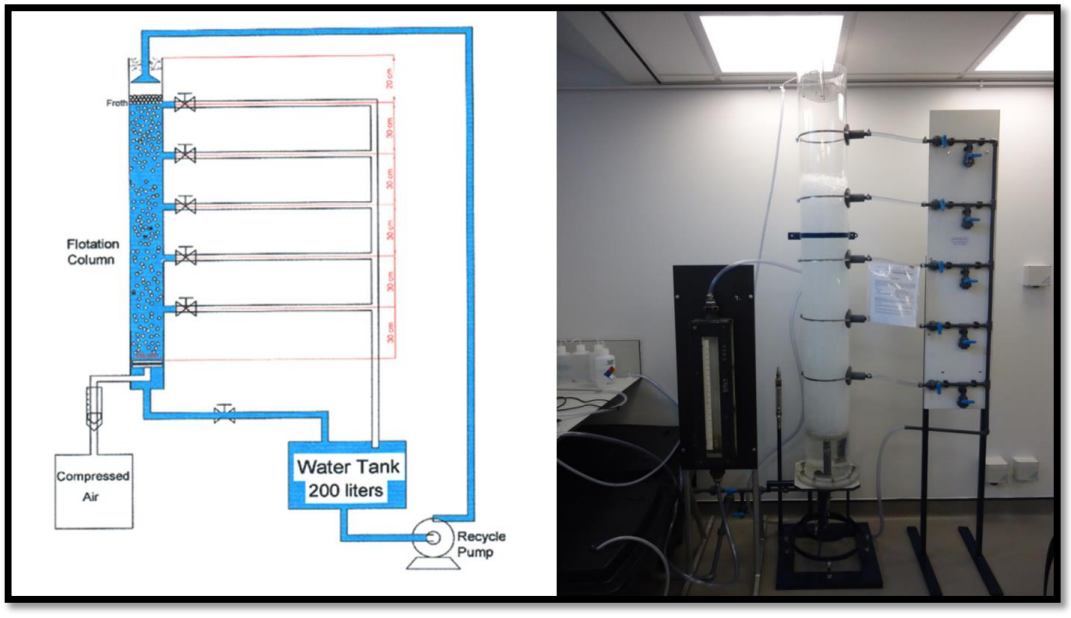


Figure 1: Experimental set up

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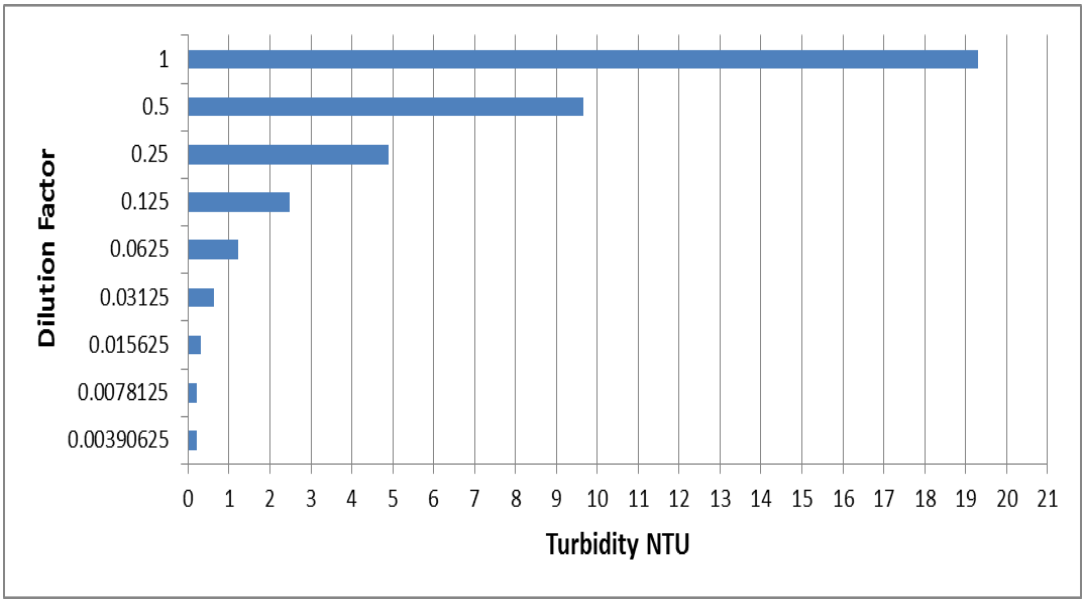


Figure 2: The relation between turbidity (NTU) and dilution factor

## 3 Operating methods

### 3.1 Batch system (without downstream)

In this set, the column is filled with a certain amount of water then starting the experiment with the same level then repeating for five definite levels.

1- Collect approximately 100 liters of distilled water in the tank.
2- Add gradually a suitable amount of cultured broth till reaching the desired turbidity (NTU) in the tank.
3- Start air pumping at a rate inside the column depending on run demands.
4- Start water pumping at a rate of 1 l/min.
5- When the froth reaches the 30 cm of column, stop upstream flow.
6- Start taking samples for turbidity reading every five minutes for 30 minutes.
7- When finished sampling for the selected time period, drain the flotation column. When fully empty, close the bottom stream.
8- Repeat steps 2 through 7 with every new air flow rate and column height.

### 3.2 Continuous System

In this set, there is an upstream and downstream. They have been adjusted so that to keep water in the column in certain levels “side streams level”.

1- Collect approximately 100 liters of distilled water in the tank.
2- Add gradually a suitable amount of cultured broth till reaching the desired turbidity (NTU) in the tank.
3- Start air pumping at a rate inside the column depending on run demands.
4- Start water pumping at a rate of 1 l/min.
5- When the froth reaches the 30 cm of column, open the bottom valve with the same flow rate as the upcoming stream.
6- Start taking samples for turbidity reading every five minutes for 30 minutes.
7- Stop upstream pumping to drain the column. When fully empty, close the bottom valve.
8- Repeat steps 2 through 7 with every new air flow rate and column height.

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## 4 Results

Air flow rate, Time, initial (tank) turbidity, and water level (side stream height) were optimized for their effect on froth turbidity. Each single graph represents the effect of air flow rate and time for different water levels (side streams height) or initial turbidity of (0.5, 1, 1.5, 2 and 2.5 NTU). Ranges for air flow rate were 10 to 170 l/min with intervals of 20 l/min. Samples were taken every five minutes. The five side streams were 30 cm apart.

Every graph represents the effect of air flow rate and time on froth turbidity. Then five graphs are developed each one is for a new water level starting from 30 cm above the sparger till 150 cm in 30 cm steps. The general trend showed an increase of froth turbidity with air flowrate till reaching the maximum level at 130 L min-1 then drop down suddenly to a value near the tank turbidity. Froth turbidity increased with time till reaching a steady level in a range up to 20 minutes. Higher water levels in the column gave greater froth turbidity. Finally, as the initial (tank) turbidity increases as the froth turbidity increases also.

### 4.1 Batch system

The effect of the three studied parameters (Air flow rate, Time, and water level) on the froth turbidity in a batch system at an initial (tank) turbidity of 2.5 NTU following the steps in (3.1) are summarized in figure 3.

### 4.2 Continuous system

The following sets were implemented according to (3.2). Every five graphs represent the effect of air flow rate, time, and water level on the froth turbidity for a given initial (tank) turbidity. Figures 4 through 8 illustrate the initial turbidities of 0.5 to 2.5 with a step of 0.5 NTU.

## 5 Discussion

The main aim of this study is to determine whether froth formed without chemical frothers and collectors can function in removing bacteria from water as a promising application in the drinking water industry. Bacteria; being hydrophobic, should be suitable for such a separation process. The role of chemical frothers is to help water and air to form froth while the role of chemical collectors is to keep the particles attached to the bubbles in the froth. However, in mineral froth flotation the optimum particle size is 88- 500 microns (Zech et al., 2012).

The separated particle, if it is collected without chemical collectors, slips down the froth due to their weight. The weight of the attached particles works against four kinds of forces, van der Waals, electrostatic, hydrodynamic repulsion, and hydrophobic forces (Bondelind et al., 2013) which hold the particle to the froth. However, much small particles; such as bacteria, provided they do not agglomerate, may not need chemicals to help attach them to froth bubbles. That small particles have a tendency to agglomerate was recently challenged when Nano Carbon Tubes were separated by froth flotation (Lertrojanachusit et al., 2013).

The effect of four variables on the turbidity (as a measure of bacterial content) of the froth was investigated. These were, air flow rate, time, water level in the column and starting (tank) turbidity. These variables were tested using complete factorial analysis where all the possibilities across all the experiments ranges were taken into account (Collins et al., 2009).

For air flow rate, froth turbidity increases proportionally along the range 10-130 l/min. Within this range, more air pumping leads to more bubbles and more chance for bubble-bacterial attachment. For the range 90-130 l/min the decrease is not sharp because of increased turbulence. At a rate of 150 l/min and more, froth is destroyed completely as a result of turbulence in the column. The mixing becomes very high and results in the bacterial concentration along the column being the same and similar to the tank concentration.

Froth turbidity is proportional to the height of the water column. When a bubble rises in a water column containing bacteria, a higher water level gives a bubble more time for bacterial–bubble attachment.

Five initial (tank) turbidities were used (0.5, 1, 1.5, 2 and 2.5 NTU). The general trend shows that the greater the initial turbidity the greater the obtained froth turbidity. These five starting values gave optimum froth turbidities of (7.23, 10.48, 16.77, 23.16 and 33.26 NTU) respectively at the optimum operating conditions for each set. These results show that the efficiency gets higher at higher initial (tank) turbidity. This is due to greater probability of bubbles to attach bacteria.

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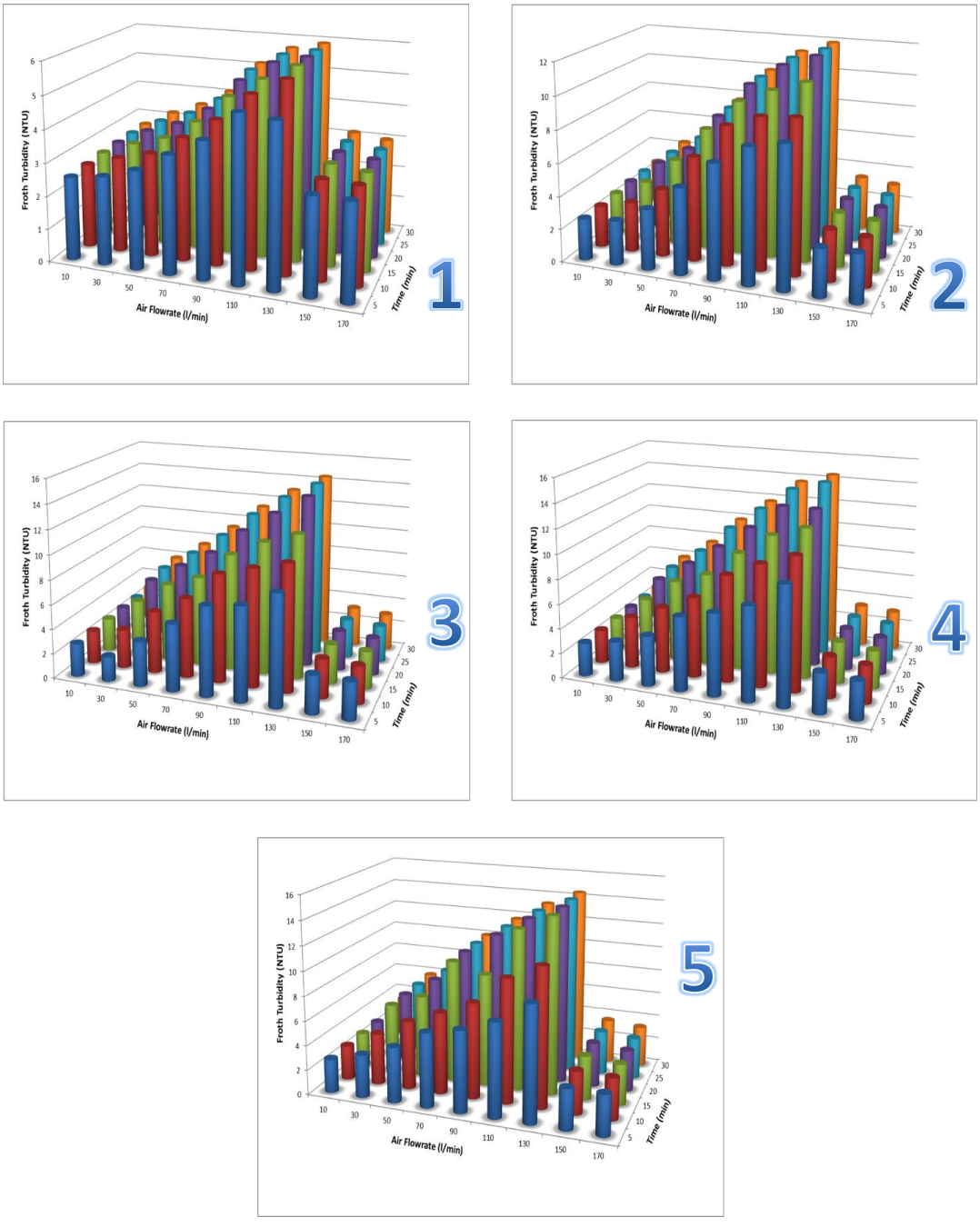


Figure 3: Effect of air flow rate and time on froth turbidity for a batch system (initial (tank) turbidity of 2.5 NTU) and for water levels of 1- 30 cm, 2- 60 cm, 3- 90 cm, 4- 120 cm, 5- 150 cm.

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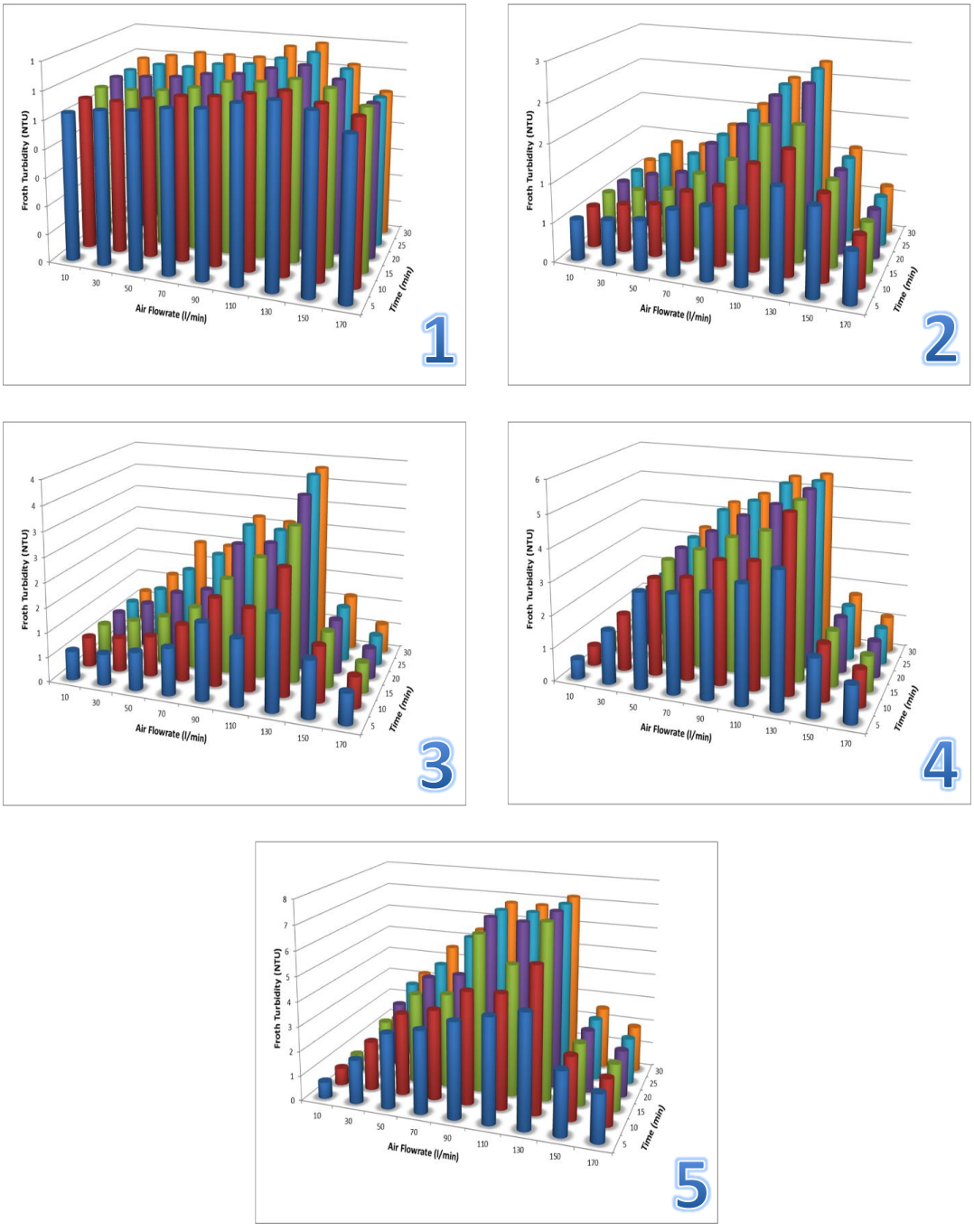


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146 Figure 4: Effect of air flow rate and time on froth turbidity for initial (tank) turbidity of 0.5 NTU and for water
147 levels of 1- 30 cm, 2- 60 cm, 3- 90 cm, 4- 120 cm, 5- 150 cm.

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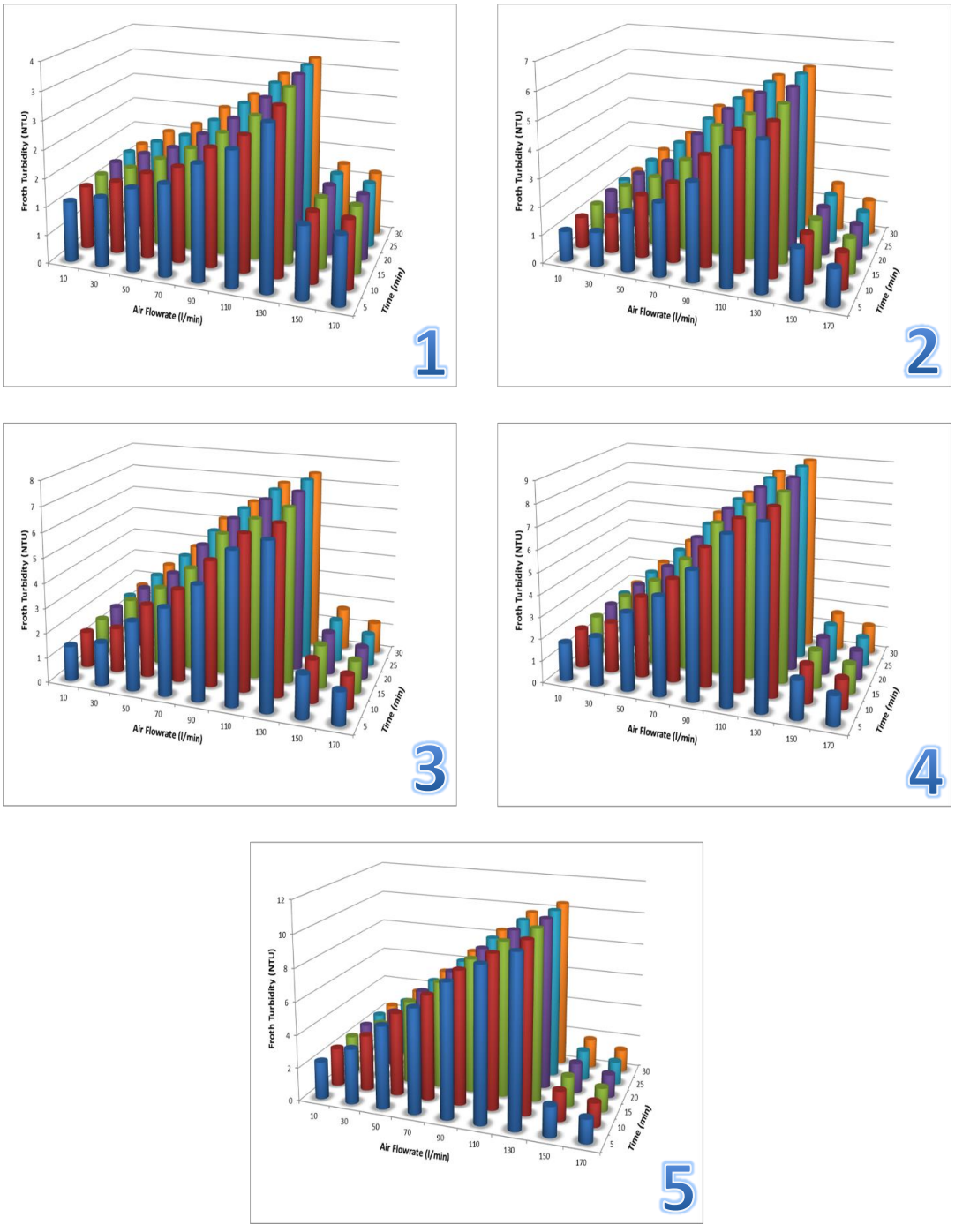


Figure 5: Effect of air flow rate and time on froth turbidity for initial (tank) turbidity of 1 NTU and for water levels of 1- 30 cm, 2- 60 cm, 3- 90 cm, 4- 120 cm, 5- 150 cm.

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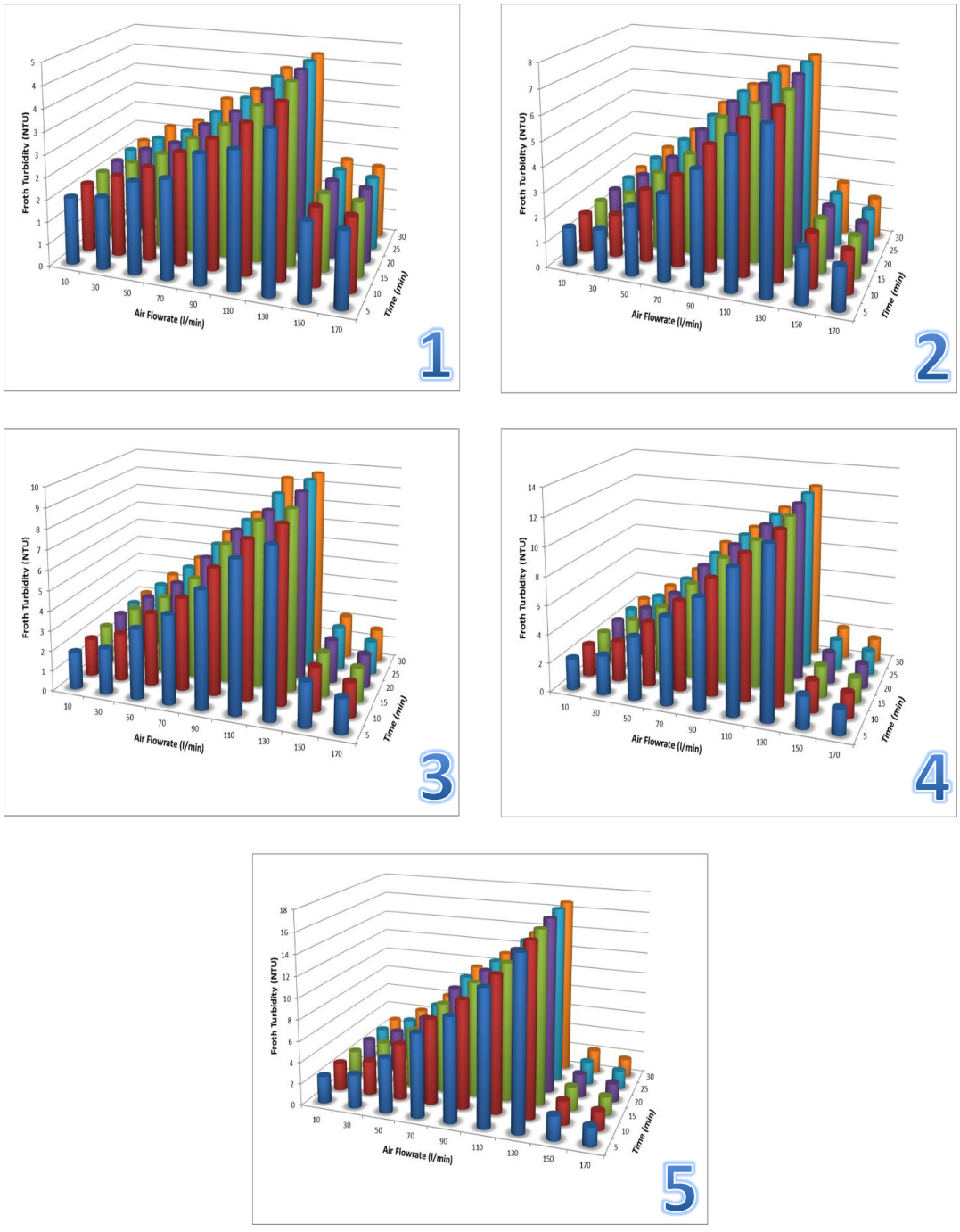


Figure 6: Effect of air flow rate and time on froth turbidity for initial (tank) turbidity of 1.5 NTU and for water levels of 1- 30 cm, 2- 60 cm, 3- 90 cm, 4- 120 cm, 5- 150 cm.

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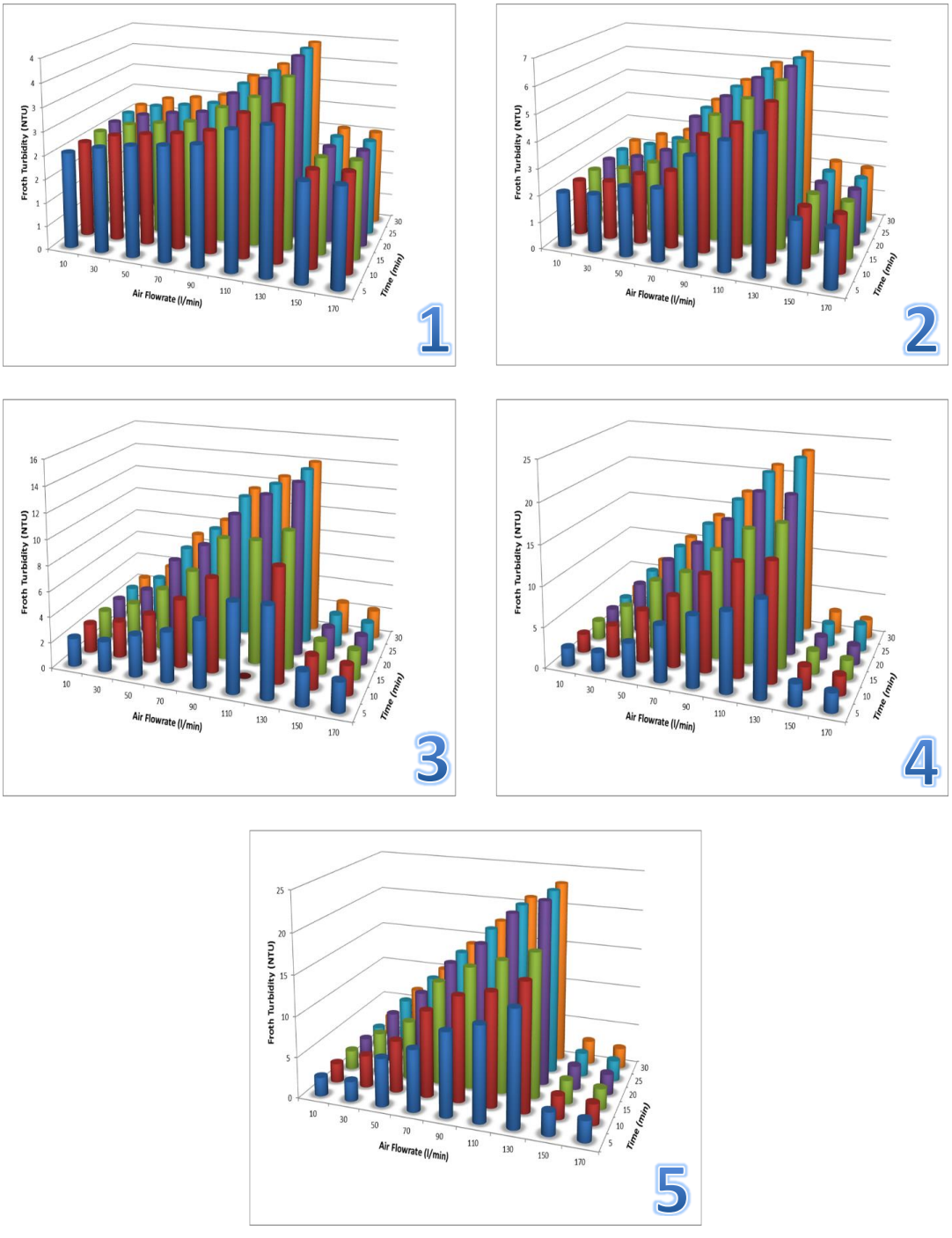


Figure 7: Effect of air flow rate and time on froth turbidity for initial (tank) turbidity of 2 NTU and for water levels of 1- 30 cm, 2- 60 cm, 3- 90 cm, 4- 120 cm, 5- 150 cm.

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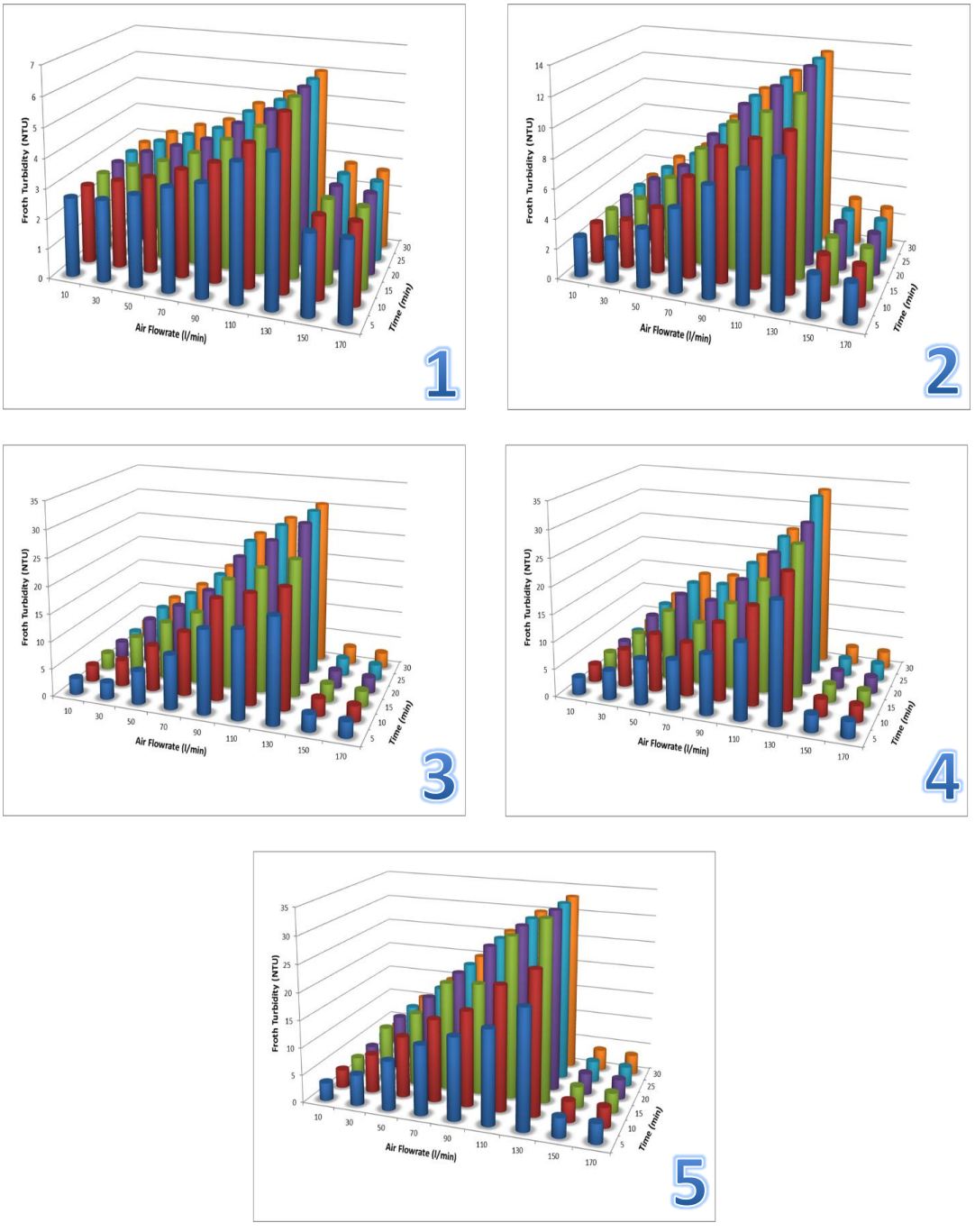


Figure 8: Effect of air flow rate and time on froth turbidity for initial (tank) turbidity of 2.5 NTU and for water levels of 1- 30 cm, 2- 60 cm, 3- 90 cm, 4- 120 cm, 5- 150 cm.



The last variable investigated was the time to reach steady froth turbidity. Froth turbidity increases until reaching a constant value. This variable is inversely proportional to air flow rate, initial turbidity and water level. The explanation of all these trends is the chance of bacteria to attach with a bubble.

These results, without the use of chemicals, are; to some extent, similar to previous researcher's findings for various applications, where chemicals were used. For instance, the first use of froth flotation with bacteria was isolating bacterial strains for laboratory purposes in the 1950's but its use disappeared with the development of more sophisticated techniques (Boyles and Lincoln, 1958;Rubin et al., 1966;Bahr and Schugerl, 1992;Rios and Franca, 1997). In mineralogy some bacteria are recognized to have two functions, attaching to minerals and being highly hydrophobic. They are found to be ideal for mineral upgrading as some minerals are not hydrophobic and cannot be otherwise separated using froth flotation (Smith et al., 1993;Nagaoka et al., 1999). The most recent application was purification of sea water in fish farms. Sea water was sucked continuously to a froth column and the bacteria removed to keep the environment healthy for the fish (Suzuki et al., 2008).

The optimum froth turbidity obtained was 33.26 NTU ($2\times10^8$ cfu/ml). The initial (tank) water stream of 2.5 NTU; ($10^7$ cfu/ml), is inputted continuously to the top of the column. Dividing these two numbers on each other gives 20. This means; theoretically, every 1 ml of froth can purify 20 ml of water completely. Practically, this needs further research. For rivers and reservoirs bacterial content could be taken as $10^4$ cfu/ml in average (Obi et al., 2003;Agbabiaka and Oyeyiola, 2012;Rajiv et al., 2012;Sakai et al., 2013) which shows promise for further work.

## 6 Conclusions

Practically, chemicals are used as frothers, collectors, activators, depressants and pH controllers that are necessary for standard froth flotation. In this study the ability of froth to separate bacteria without any of these associated chemicals was investigated. The results show that the separation force of froth alone is sufficient for bio purification. These results indicate the potential to move towards water treatment with lower or no biocides.

The findings of this work widen the horizon for many applications. The first is drinking water treatment. Bacterial and other solids concentration can be lowered to an acceptable range either by this treatment alone or as an introduction to other purification steps. Food and pharmaceutical industries are other fields for such applications. It can be used as an alternative, or in series, with filtration and sedimentation for decreasing bacterial and solid content.

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