

# WAIHI BEACH WASTEWATER TREATMENT PLANT – SOME EFFECTS OF HIGH SPEED PUMPING AND MIXING ON ACTIVATED SLUDGE BACTERIA

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## ABSTRACT

The Waihi Beach treatment plant, constructed in 2001 for Western Bay of Plenty District Council (WBOPDC), consists of a single-lagoon sequential batch reactor (SBR) followed by a wetland and a UV disinfection plant. Historically the plant struggled to provide enough aeration during peak periods. A new 35 year resource consent required a review of the plant and professional advisors had mixed views about the preferred re-design of the aeration system.

This paper is an on-site study of the treatment plant aeration system and how it is affecting the activated sludge process. The purpose of this paper is to assist the WBOPDC in making a more informed decision about the aeration upgrade.

Studies of the existing system found that prolonged use of the turbo-aerators caused damage to the activated sludge floc. Damage was measured using effluent turbidity and disrupted heterotrophic bacteria. The effluent was still compliant with discharge requirements. Loss of these bacteria in the effluent is considered to be irreversible and undesirable.

A number of simulated high speed pumping tests showed that the critical factor causing permanent disruption of heterotrophic bacteria from the sludge floc was fluid velocity or speed. After three passes at the slowest pumping speed of  $29 \text{ m sec}^{-1}$  the heterotrophic bacteria were easily disrupted (70 to 80%). Disruption of the sludge floc released more Gram negative bacteria than Gram positive ones and the "viable" bacteria could not be re-aggregated with the floc by gentle mixing. More than half the "non-viable" bacteria and non-bacterial particles were re-aggregated.

It is recommended that upgrading the Triton mixer/blower units rather than turbo-aerators is a better strategy.

## KEYWORDS

**wastewater, activated sludge, floc damage, high speed pump, surface aerator, heterotrophic bacteria**

## 1 INTRODUCTION

The Waihi Beach wastewater treatment plant was designed and built in 2001 with a single aeration lagoon operating as an activated sludge sequential batch reactor (SBR). Oxygen is provided by two high speed surface aerators (turbo-aerators) and four slow speed mixer/blower combinations (Tritons). Photograph 1 shows one of the two turbo-aerators operating at the plant and photograph 2 shows an inverted view of the impellor.

Secondary treatment of influent sewage from approximately 2,000 people is carried out in the SBR lagoon with a working volume of approximately  $7,500 \text{ m}^3$ . The township is a popular holiday destination and so the population can vary up to 5,000 or more. The influent volumes can vary considerably between 600 and  $3,000 \text{ m}^3$  per day.

In the past, the aeration system has been unable to cope with the demand for oxygen for short periods when large numbers of holidaymakers visited the area. For the time being this problem has been resolved with the addition of two more medium speed surface aerators. However it is likely that more aeration will be needed in the medium term as the numbers of visitors to the area rise especially during the summer holidays.

Following aeration the effluent is decanted into a constructed wetland, pumped through an ultraviolet disinfection plant, flowing on through a small flood-irrigation area and discharging into a small creek. The creek flows through the main township and on to the local beach.

*Photograph 1: One of the turbo-aerators at Waihi Beach treatment plant*



*Photograph 2: Inverted view of spare turbo-aerator impellor*



After being granted a new 35 year resource consent, WBOPDC is required to review the treatment plant operating efficiency and discharge conditions in response to expected population growth over the next 35 years. During the process of the current review the aeration system has been identified as requiring an upgrade. Different professional opinions have been obtained and different aeration designs have been put forward. More high speed surface aerators were the cheapest option. Low speed surface aerators were the next cheapest.

There are mixed opinions about the direction that the upgrade should take and so this prompted an on-site investigation of existing aerators.

This paper is an on-site study of the treatment plant aeration system and how it is affecting the activated sludge process. The purpose of this paper is to assist WBOPDC in making a more informed decision about the aeration upgrade.

It was sometimes observed by operators that prolonged use of the turbo-aerators resulted in some activated sludge floc damage and decanted effluent that had noticeably increased turbidity. Prolonged use of the Tritons did not have this effect. The focus for the on-site study was to measure the extent of the floc damage and then determine if the damage could be repaired or reversed and what recommendations could be made about the plant.

Some background research on this subject has provided good information on harsh physical treatment such as high speed pumping, mixing and aerating and how it increases shear rate which leads to floc breakage as either erosion or fragmentation (Jarvis et al., 2005). The reverse process of microbial attachment or aggregation occurs under mild shearing conditions. Liu and Tay established how mild shear conditions promote attachment of the microbial cells (Liu and Tay, 2002). Mild shear of 400 rpm to 600 rpm results in larger floc size and a lower sludge volume index (Liu et al., 2005). Little is known about what is actually happening to the sludge floc during this kind of physical treatment. The information that is lacking concerns the amount of shearing force that encourages flocs to form instead of breaking down. Information is also lacking on whether damaged floc can be re-aggregated and whether live and dead bacteria behave in the same manner during aggregation and breakage.

In this study it was decided to measure the floc damage in three ways. The heterotrophic plate count was used to enumerate bacteria released or disrupted from the floc. The turbidity of the gravity-settled decanted effluent or supernatant was used as an indicator of damage and the disruption of the total number of bacteria was estimated using a direct microscopic counting method.

Those colony forming units that are recovered on standard plate count agar after aerobic incubation at 28<sup>0</sup>C for 96 hours are called “viable” heterotrophic bacteria in this investigation. “Non-viable” bacteria mean those bacteria that are observed microscopically but are not recovered on standard plate count agar under the conditions that are used.

## **2 METHODS & MATERIALS**

### **2.1 SAMPLE COLLECTION**

Samples of fresh mixed liquor were collected from the activated sludge lagoon during the late aeration phase in 10 litre plastic buckets that had been washed with hot soapy water and rinsed several times with chlorine disinfected tap water.

### **2.2 WATER BLASTER TREATMENT**

The pump that was used was a commercial water-blaster fitted with a variety of different nozzles and operated at two different speed settings (“full speed” and “idle speed”). The water-blaster was a Annovi Reverberi Model XMV 3G25 (Annovi Reverberi SpA, Modena, Italia). It was powered by a Honda 5.5HP GX160 petrol engine. The water-blaster was connected to a 20 litre drum that allowed 8 litres of mixed liquor sample to gravity-feed into the pump. Using the water-blaster hydraulic hose, gun and nozzle the liquor was recycled back into the drum several times. Between samples the drum and pump were rinsed with disinfectant and chlorinated tap water.

## **2.3 BREVILLE BLENDER**

250cm<sup>3</sup> mixed liquor samples were homogenised in a 750 watt Breville “Light & Mighty” food blender (Breville Group Ltd, Botany, NSW, Australia) for various times up to 2.5 minutes. Samples blended for longer were cooled in a refrigerator for 20 minutes after blending for 2.5 minutes and then blended longer for up to 2.5 minutes. This procedure prevented the temperature of the mixed liquor rising above 37 °C.

## **2.4 SETTLING TESTS**

Glass test tubes were filled with mixed liquor and allowed to settle by gravity for 30 minutes. They were then photographed so that a qualitative record of sludge quantity and supernatant (effluent) clarity could be kept. Progressive settling of sludge can be seen in photographs 3, 4 & 5.

## **2.5 TURBIDITY TESTS**

The turbidity of effluent or supernatant was determined using a HACH 2100P turbidity meter (Thermo Fisher Scientific New Zealand, 244 Bush Road, Albany, Auckland). Samples of mixed liquor were allowed to settle for 30 minutes before decanting the supernatant. The turbidity of the supernatant was measured immediately. The instrument was calibrated in Nephelometric Turbidity Units (NTU) using 0.05 NTU, 20.1 NTU, 100 NTU and 803 NTU stabilised Formazin reference standards Lot A2117 provided by HACH Company, Loveland, CO, USA.

## **2.6 HETEROTROPHIC PLATE COUNT (HPC)**

Heterotrophic plate counts were determined using the reference method in Standard Methods for the Examination of Water and Wastewater, 22<sup>nd</sup> Edition 2012 (American Public Health Assoc., I Street NW Washington DC., USA). Ten fold serial dilutions made in 0.1% peptone water were plated on Plate Count Agar and incubated at 28°C for 96 hours. Duplicate plates with 30 to 300 colonies were counted and recorded. Bacteriological Peptone and Plate Count Agar were obtained from Oxoid Ltd, Hampshire, UK.

Media and glassware were sterilised on site using a Prestige kitchen pressure cooker (Meyer Group, Bromborough, Wirral, UK) at 15 psi steam pressure for 20 minutes. Disposable, gamma sterilised transfer pipettes and petri dishes were obtained from Thermo Fisher Scientific New Zealand, Albany, Auckland.

## **2.7 GRAM STAINS**

Gram stains were carried out using a Remel Gram staining kit (Remel House, Dartford, UK). Slides were flooded with crystal violet for 2 minutes then iodine mordant for 1 minute. Acetone decolouriser was applied for 3 to 5 seconds rinsed off immediately with tap water prior to flooding the slide with safranin counterstain for 1.5 minutes. Excess safranin was washed off with tap water and the slides were dried using paper towels and a hair drier.

## **2.8 DIRECT MICROSCOPIC COUNT (DMC)**

The DMC used in this study is a count of bacterial particles, including single cells, clumps and chains that could potentially grow and form a colony if they were all viable. The clumps (includes flocs) and chains are counted as one. It is not a count of individual cells.

The microscope that was used is an XJS304 trinocular bright field microscope manufactured by Nanjing Kozo Optical and Electronic Instruments, Nanjing, China. It was fitted with a Pro-Microscan DCM130E, 5 Megapixel digital camera manufactured by Oplenic Optronics Hangzhou, Zhejiang, China. The field diameter of the 100 times objective lens was determined using a 1 mm stage micrometer graduated in 1, 5 and 10 µm divisions. The field diameter of the lens was 198µm. The number of fields per cm<sup>2</sup> was 3,247 and the multiplying factor for one single particle per field was 324,731. A 10µL sample of mixed liquor was spread over a 1cm by 1cm square area on a glass slide. The sample was gently heat-fixed over a burner, Gram stained, dried and examined under the microscope using oil immersion at 1,000 times magnification. Representative fields were counted. The number of cells, clumps or chains was determined using the multiplying factor. Each separate cell, clump or chain was counted as one. Whole or partial floc particles were also counted as one.

### 3 RESULTS

#### 3.1 TREATMENT PLANT HPC & EFFLUENT TURBIDITY

The turbo aerators turn on and off at pre-determined levels of dissolved oxygen. On 12<sup>th</sup>, 13<sup>th</sup> and 14<sup>th</sup> December 2013 effluent turbidity, HPC and aerator running times were measured during the day time cycle. There were few holiday visitors in town. This was repeated on 31<sup>st</sup> December 2013, 1<sup>st</sup> January 2014 and 2<sup>nd</sup> January 2014 when there were many holiday visitors in town. The results are presented in Table 1. In the first group of samples in mid-December the turbo-aerator times were 23 to 28 minutes. The average effluent turbidity was 4.1 NTU and the average HPC was  $7.2 \times 10^5$  cfu cm<sup>-3</sup>. In the second group of samples aerator times were 440 to 663 minutes. The average turbidity of the decanted effluent increased to 10.6 NTU. The average HPC increased four fold up to  $2.9 \times 10^6$  cfu cm<sup>-3</sup>.

Table 1: Effect of Aeration Time on HPC and Effluent Turbidity

Date	Turbo Aerator Minutes	Effluent Turbidity NTU	Effluent HPC* cfu cm <sup>-3</sup>
12/12/2013	23	3.7	$7.30 \times 10^5$
13/12/2013	28	4.4	$8.80 \times 10^5$
14/12/2013	25	4.1	$5.60 \times 10^5$
31/12/2013	663	9.3	$3.20 \times 10^6$
01/01/2014	436	13.1	$1.25 \times 10^6$
02/01/2014	440	10.6	$4.25 \times 10^6$

\* Heterotrophic Plate Count

These results show that when the turbo-aerators are used extensively there is a four fold increase in the heterotrophic bacteria lost in the effluent and a small but noticeable increase in effluent turbidity.

#### 3.2 PUMPING SPEED TESTS

When high speed pumping was simulated using a water-blaster, four different sized nozzles and two different pump speeds were used. Water velocities ranging from 29 to 142 m sec<sup>-1</sup> were achieved.

Water velocities have been determined for each nozzle by pumping tap water at maximum engine speed into a large drum. Slower velocities were achieved by pumping water at idling speed. The water was gravity-fed into the water-blaster from a 20 litre drum. The volume of water collected in a 100 litre drum after 20 seconds was measured. Nozzle sizes of 1.285, 1.796, 2.295 and 3.224 mm<sup>2</sup> were determined using a microscope and stage micrometer. At full engine speed water velocities of 142, 110, 88, and 66m sec<sup>-1</sup> were achieved. At idling engine speed water velocities were 66, 50, 39 and 29m sec<sup>-1</sup>.

#### 3.3 MIXED LIQUOR STABILITY BEFORE & AFTER PUMPING

Samples of mixed liquor were taken before and after high speed pumping for three cycles at 88 m sec<sup>-1</sup>. The sample before pumping was divided into two samples. One of these samples was allowed to settle for 30 minutes and the top layer poured into a test tube. This is sample B in photographs 3, 4 & 5. The other sample of untreated liquor was inverted three times prior to testing and poured into another test tube. This is sample A in the photographs. The treated sample was also divided into two separate samples, one of them allowed to settle and the other poured into a test tube after inverting three times just prior to the test period. Sample C is the treated sample and sample D is the supernatant layer of the duplicate treated sample.

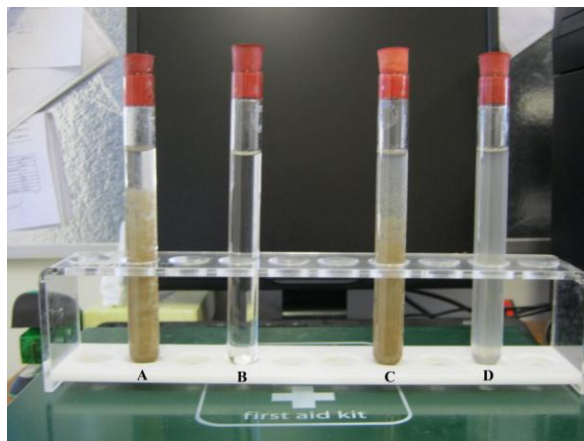
All of the samples had been gently mixed simultaneously by inverting three times just prior to the 30 minute test period. Photographs 3, 4, and 5 show progressive settling of sludge over a 30 minute time period in samples A and C. There is no settling in samples B and D. Samples A and C in photograph 5 show sludge layers which have separated from the supernatant. The untreated sample A has a clear supernatant layer while the treated sample C has a turbid supernatant layer. Separate samples of both supernatants were decanted off other identical

samples just prior to this settling test. These are shown in samples B and D. Both B and D samples are unchanged. Even several hours later both the clear decanted supernatant and the turbid decanted supernatant are unchanged. When the amount of settled sludge in sample A (untreated) is compared to sample C (after pumping) approximately one third of the total has been disrupted and dispersed into a relatively stable suspension.

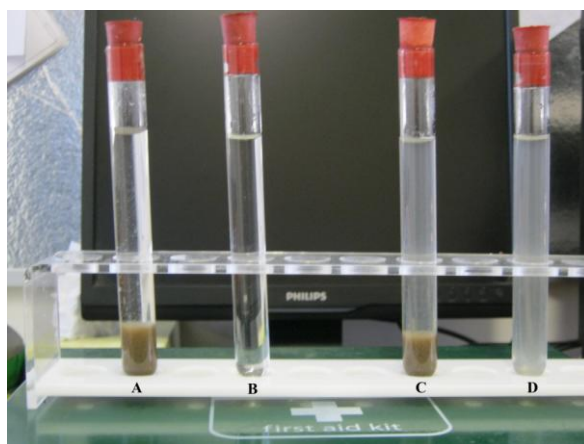
*Photograph 3: Stability Tests – Immediately After Mixing*



*Photograph 4: Stability Tests – 15 Minutes After Mixing*



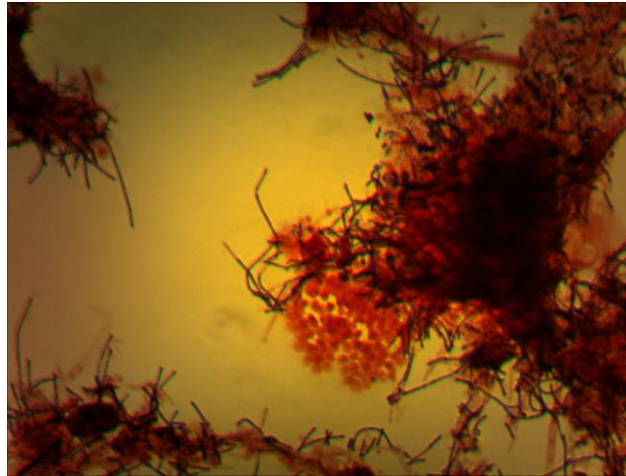
*Photograph 5: Stability Tests – 30 Minutes After Mixing*



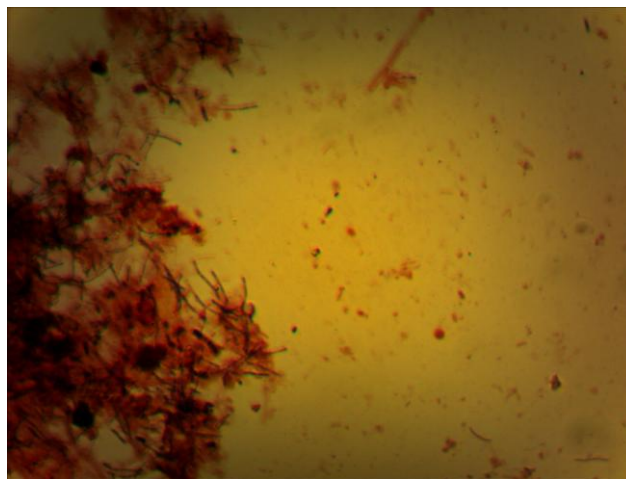
A typical Gram stain of the bacteria in fresh untreated mixed liquor is shown in photograph 6. It illustrates many gram positive, filamentous bacteria making up part of the fibrous structure of the activated sludge floc at Waihi Beach. Inside this random structure are many more Gram negative and some other Gram positive bacteria. Outside of this structure there are few if any individual bacteria present. Photograph 7 is a typical Gram stain of mixed liquor immediately after three passes of high speed pumping treatment.

It shows a partially damaged sludge floc made of Gram positive and Gram negative bacteria. Outside of the sludge floc are numerous individual particles including many whole Gram negative bacteria and some Gram positive bacteria. There are many other weakly stained but visible bacterial rods and cocci. Clumps and chains were counted as one particle. Particles that were too small, almost invisible or wrongly shaped were not counted in the DMC. The magnification is 1,000 times for both photographs.

*Photograph 6: Fresh Mixed Liquor – Gram Stain (Sample A)*



*Photograph 7: Mixed Liquor After Pumping – Gram Stain (Sample C)*



### **3.4 HPC, DMC & EFFLUENT TURBIDITY BEFORE & AFTER PUMPING**

#### **3.4.1 HIGH SPEED PUMPING**

After pumping fresh samples of mixed liquor three times at 66, 88, 110 and 142 m sec<sup>-1</sup> (Table 2), HPC increased 5.8, 7.4, 7.6 and 7.3 times respectively. DMC increased 31, 73, 95 and 124 times respectively and the turbidity of the decanted effluent increased from 6 to 44, 173, 215 and 270 NTU respectively.

Table 2: HPC, DMC & Effluent Turbidity Before & After High Speed Pumping

Nozzle Size mm <sup>2</sup>	Velocity m sec <sup>-1</sup>	ML HPC * cfu cm <sup>-3</sup>	ML DMC ** cfu cm <sup>-3</sup>	Effluent Turbidity NTU
Untreated	0	8.0 x 10 <sup>5</sup>	5.5 x 10 <sup>6</sup>	6
3.224	66	4.6 x 10 <sup>6</sup>	1.7 x 10 <sup>8</sup>	44
2.295	88	5.9 x 10 <sup>6</sup>	4.0 x 10 <sup>8</sup>	173
1.796	110	6.1 x 10 <sup>6</sup>	5.2 x 10 <sup>8</sup>	215
1.285	142	5.8 x 10 <sup>6</sup>	6.8 x 10 <sup>8</sup>	270

\* Heterotrophic Plate Count  
 \*\* Direct microscopic count of bacterial cells, clumps or chains

### 3.4.2 SLOWER SPEED PUMPING

After pumping mixed liquor five times at 29, 39, 50 and 66 m sec<sup>-1</sup> (Table 3), HPC increased 4.0, 5.2, 4.6 and 5.4 times respectively. DMC increased 24, 41, 44 and 36 times respectively and the turbidity of the decanted effluent increased from 5 to 37, 67, 80 and 122 NTU respectively. Table 3 shows that 70 to 80% of the "viable" heterotrophic bacteria and 55 to 65% of the "non-viable" bacteria are disrupted from the sludge flocs at the slowest pump velocity or speed of 29 m sec<sup>-1</sup>.

Table 3: HPC, DMC & effluent turbidity before & after slower speed pumping

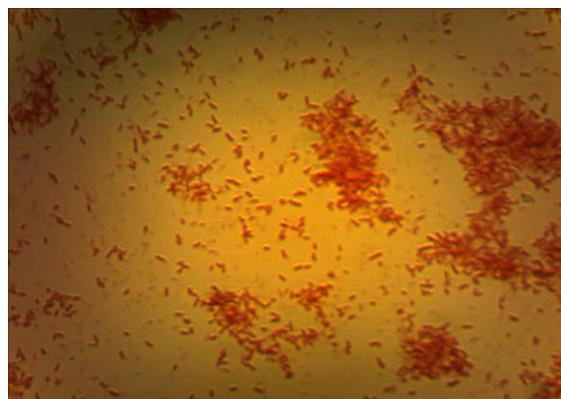
Nozzle Size mm <sup>2</sup>	Velocity m sec <sup>-1</sup>	ML HPC * cfu cm <sup>-3</sup>	ML DMC ** cfu cm <sup>-3</sup>	Effluent Turbidity NTU
Untreated	0	8.1 x 10 <sup>5</sup>	1.6 x 10 <sup>6</sup>	5
3.224	29	3.2 x 10 <sup>6</sup>	3.9 x 10 <sup>7</sup>	37
2.295	39	4.2 x 10 <sup>6</sup>	6.5 x 10 <sup>7</sup>	67
1.796	50	3.7 x 10 <sup>6</sup>	7.1 x 10 <sup>7</sup>	80
1.285	66	4.4 x 10 <sup>6</sup>	5.8 x 10 <sup>7</sup>	122

\* Heterotrophic Plate Count  
 \*\* Direct microscopic count of bacterial cells, clumps or chains

### 3.5 PREDOMINANCE OF GRAM NEGATIVE RODS AFTER PUMPING

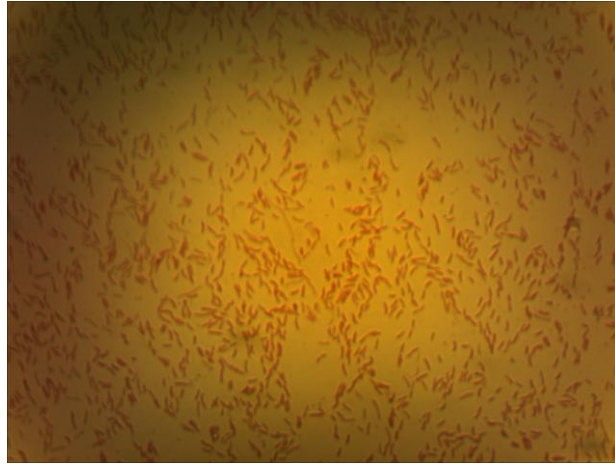
Gram stains of mixed liquor after high speed pumping showed an abundance of Gram negative rods that are released by this process. Colonies that were randomly picked off agar plates also showed a predominance of Gram negative rods along with a few Gram positive cocci (photographs 8, 9 & 10).

Photograph 8: Gram Negative Rods Isolated From Waihi Beach WWTP

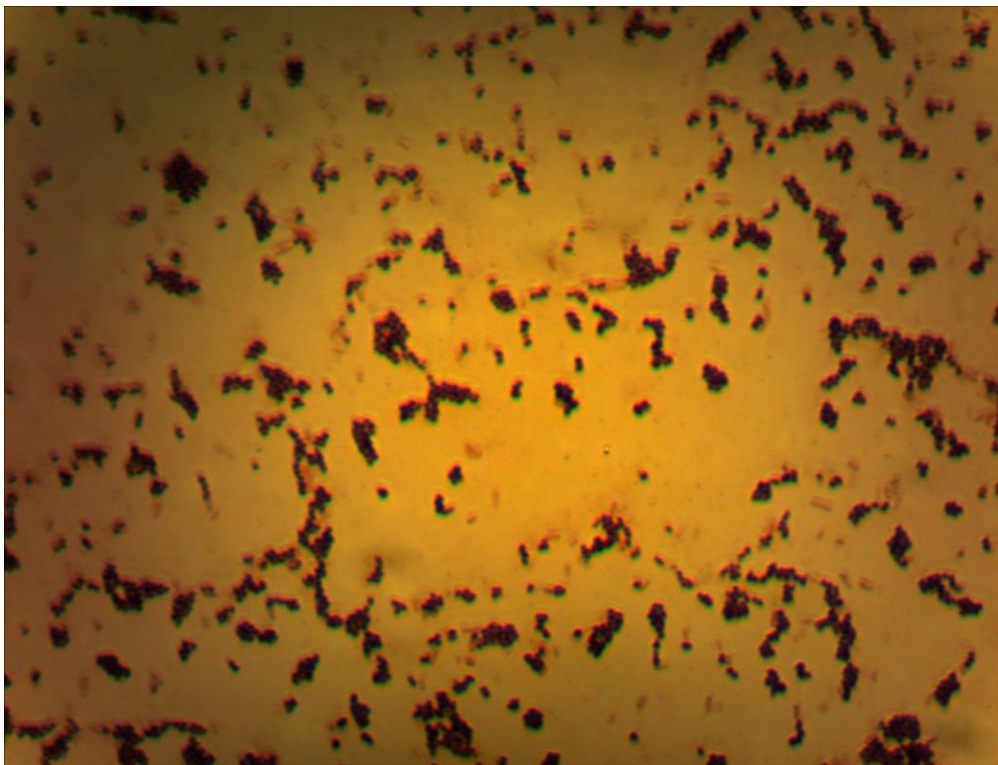




*Photograph 9: Gram Negative Rods Isolated From Waihi Beach WWTP*



*Photograph 10: Gram Positive Cocci Isolated From Waihi Beach WWTP*



### **3.6 HPC, DMC & EFFLUENT TURBIDITY AFTER BLENDING**

Fresh mixed liquor samples were processed in a Breville food blender to simulate high speed mixing and shearing forces (Table 4).

Five separate sub-samples of the same mixed liquor were blended for various times. Blending times used were 15, 30, 60, 150, and 300 seconds. The sample that was blended for 300 seconds was interrupted at 150 seconds and allowed to cool in a refrigerator for twenty minutes. Blending then continued for a further 150 seconds.

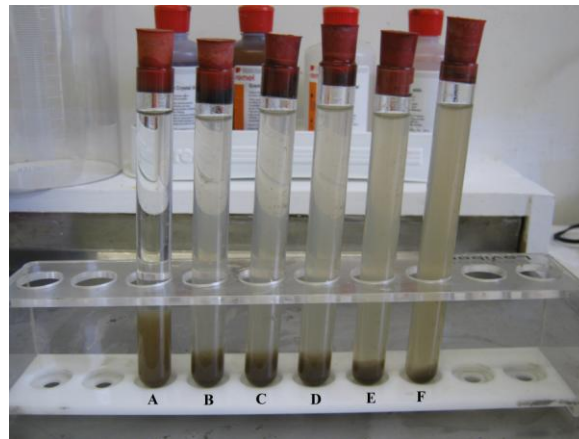
After blending for 15, 30, 60, 150 and 300 seconds: HPC increased 8, 13, 8, 11 and 11 times respectively. DMC increased 17, 19, 32, 85 and 143 times respectively. Turbidity increased from 13 NTU to 91, 110, 147, 222 and 349 NTU respectively. After 300 seconds of blending there is only a small amount of sludge left (sample F, photograph 11). The amount left over is approximately 10 to 20% of the original.

Table 4: HPC, DMC & Effluent Turbidity Before & After Blending

Blender Times (seconds)	ML HPC * (cfu cm <sup>-3</sup> )	ML DMC ** (cfu cm <sup>-3</sup> )	Effluent Turbidity (NTU)
Untreated	1.0 x 10 <sup>6</sup>	8.4 x 10 <sup>6</sup>	13
15	7.9 x 10 <sup>6</sup>	1.4 x 10 <sup>8</sup>	91
30	1.3 x 10 <sup>7</sup>	1.6 x 10 <sup>8</sup>	110
60	8.4 x 10 <sup>6</sup>	2.7 x 10 <sup>8</sup>	147
150	1.1 x 10 <sup>7</sup>	7.1 x 10 <sup>8</sup>	222
300	1.1 x 10 <sup>7</sup>	1.2 x 10 <sup>9</sup>	349

\* Heterotrophic Plate Count  
 \*\* Direct microscopic count of bacterial cells, clumps or chains

Photograph 11: Mixed Liquor After Blending For Various Times Then Allowed To Settle



### 3.7 RE-MIXING BLENDER DAMAGED LIQUOR

In Table 5, fresh liquor that had been blended for 1 minute was subjected to gentle re-mixing for 30 minutes on a laboratory magnetic stirrer. HPC of the damaged liquor and the re-mixed liquor were the same. The DMC result was 2.6 x 10<sup>8</sup> cfu cm<sup>-3</sup> for the damaged liquor and 1.1 x 10<sup>8</sup> cfu cm<sup>-3</sup> (58% less) for the re-mixed liquor. The decanted effluent of the damaged liquor had a turbidity of 213 NTU and the re-mixed liquor, 55 NTU (75% less) respectively.

These results show that sludge floc from the treatment plant when damaged by blending was partially re-assembled by gentle mixing. However, because the HPC results were the same after disruption and after gentle mixing it is concluded that the heterotrophic bacteria did not re-attach to new or existing floc particles.

Table 5: HPC, DMC & effluent turbidity after blending and re-mixing

Sample Treatment	ML HPC * (cfu cm <sup>-3</sup> )	ML DMC ** (cfu cm <sup>-3</sup> )	Effluent Turbidity (NTU)
Untreated	5.7 x 10 <sup>5</sup>	6.1 x 10 <sup>5</sup>	7
Blended 1 min	4.9 x 10 <sup>6</sup>	2.6 x 10 <sup>8</sup>	213
Re-mixed 30 min	4.9 x 10 <sup>6</sup>	1.1 x 10 <sup>8</sup>	55

\* Heterotrophic Plate Count  
 \*\* Direct microscopic count of bacterial cells, clumps or chains

## 4 CONCLUSIONS

When the aeration system at the Waihi Beach treatment plant was examined more closely it was found that the turbo-aerators in particular were causing some damage to the activated sludge floc when their use was prompted by greater demands for oxygen. Prolonged use of the turbo-aerators resulted in minor damage as measured by an increase in effluent turbidity of more than two fold and a four fold increase in the number of heterotrophic bacteria in the effluent. However the effluent was still compliant with discharge requirements.

Further investigation at the treatment plant laboratory provided more in depth information about the nature and extent of sludge floc damage after simulated high speed pumping and mixing using a water-blaster and a food blender. For example the damage inflicted on the sludge floc as measured by increasing HPC, DMC or turbidity in the effluent, was more complete at higher fluid velocities or speeds and after more exposure to the high velocity.

The high speed pumping trial that was carried out at speeds from 66 to 142 m sec<sup>-1</sup> resulted in a 5 to 7 fold increase in HPC, 30 to 124 times the original DMC and a 7 to 46 fold increase in effluent turbidity. The slower speed trial with pumping speeds from 29 to 66 m sec<sup>-1</sup> showed a 4 to 5 fold increase in HPC, a 20 to 40 fold increase in DMC and 6 to 24 fold increase in effluent turbidity.

Five passes at the slowest pumping speed of 29m sec<sup>-1</sup> still caused extensive disruption (70 to 80%) of the "viable" bacteria. Due to the limitations of the pumping equipment 29 m sec<sup>-1</sup> was the slowest pumping speed achievable and it is most likely that speeds lower than 29 m sec<sup>-1</sup> cause some damage given sufficient exposure time. Approximately 55 to 65% of the "non-viable" bacteria were disrupted at 29 m sec<sup>-1</sup>.

Although large numbers of sludge bacteria were relatively easy to dislodge from the floc, the majority of the physical sludge floc itself was far more resistant requiring at least five minutes in a food blender to break up 80 to 90 per cent of the settleable solids. All of the "viable" bacteria were disrupted after only 30 to 60 seconds of blending.

This study has also provided good evidence that more than half of the particles disrupted into a stable suspension by high speed treatment were re-aggregated with either new or existing floc particles when the damaged liquor was subjected to gentle mixing for 30 minutes. However there is sufficient evidence to conclude that the "viable" heterotrophic bacteria did not re-aggregate at all. Their disruption was permanent. It is probably safe to conclude therefore, that most of the "viable" bacteria disrupted from the floc by the turbo-aerators at the Waihi Beach treatment plant, are lost in the decanted effluent and that this loss is irreversible. Because heterotrophic bacteria possess many attributes that enable them to metabolise and assimilate a wide array of pollutants, their loss in the effluent is considered to be undesirable.

After high speed treatment of mixed liquor in the laboratory the supernatant that was decanted off settled samples showed that the particles in suspension were stable and did not demonstrate any further settling over night. The suspension also had strong turbidity. It was concluded that the majority of this turbidity was most likely due to non-bacterial particles. The "non-viable" bacteria probably made up the next largest group of particles followed by a much less significant group of "viable" bacteria.

Large numbers of "non-viable" bacteria were released after high speed pumping of Waihi Beach liquor in the laboratory. The composition of "non-viable" bacteria was similar to the composition of "viable" bacteria released where there were predominantly Gram negative rods and a few Gram positive cocci.

## 5 RECOMMENDATIONS

As a result of this study it can be recommended to the WBOPDC that any further upgrading of the aeration should consider upgrading the slower speed mixer/blower units rather than the turbo-aerators. When there is a requirement to run the turbo-aerators for prolonged periods then the turbidity in the effluent will be less if a gentle mixing period is used just prior to the settling phase. The heterotrophic bacteria lost in the effluent could be partially compensated for by retaining more sludge.

## 6 DISCUSSION

The pumping equipment used in this study was limited. Pumping speeds slower than  $29 \text{ m sec}^{-1}$  were not achievable. Given that a speed of  $29 \text{ m sec}^{-1}$  caused 70-80% disruption of live bacteria then speeds slower than this are also going to cause at least some disruption with sufficient exposure time. It is well known however, that speeds much slower than this, instead of causing disruption, give rise to mild shear conditions and encourage floc formation and aggregation (Liu et al., 2005). The finding that speeds greater than  $29 \text{ m sec}^{-1}$  cause almost complete disruption of these bacterial clumps is a similar result to a previous study of heterotrophic, clump-forming, Gram negative psychrotrophs growing in continuously agitated, refrigerated milk (Te Whaiti I E & Fryer T F, 1977). It was found that a minimum fluid velocity of  $30 \text{ m sec}^{-1}$  was required to completely break up and properly enumerate the psychrotrophs growing as clumps in the milk.

When the activated sludge floc at Waihi Beach was disrupted, Gram negative rods out-numbered filamentous and other Gram positive bacteria. The majority were clump-forming, possessing sticky extra-cellular material that is most likely able to promote adherence to sludge floc. Prior to enumerating or isolating clump or chain forming bacteria, especially in solid or particulate matter, it is standard practice in many industries to break up the particles using blenders, homogenisers or sonicators. In the water industry, where bacterial flocs are present it is sometimes ignored but it is becoming more recognised in recent times. In 2006 Falcioni et al recommended five 45 second cycles in a 23,000 rpm tissue homogeniser followed by two 45 second cycles using a sonicator. However sonicators are well known to cause cellular disruption and death (Foladori et al., 2006) especially when used at high energy levels (Kakhruddin A N M., and Rashid H., 2005). In this study it was decided to measure the numbers of bacteria released after pumping using a water-blaster or after homogenisation using a food blender.

In 2012 Porot et al., re-defined the life and death status of bacterial cells in activated sludge and other environmental situations. They proposed that live cells have membrane integrity, normal gene expression, cell division, protein synthesis and metabolic activity. In this Waihi Beach study the bacteria were called "viable" if they recovered and divided enough to form a visible colony under the conditions of the heterotrophic plate count. They were called "non-viable" if they were not able to do that. The author is fully aware that some of the common sludge bacteria are not recoverable using the heterotrophic plate count and may prefer anaerobic or more fastidious conditions. It is believed however, that the sludge at Waihi Beach when subjected to high fluid velocities, released large numbers of heterotrophic bacteria which then out-numbered other anaerobic or more fastidious bacteria.

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