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Maximum HPC As a Measure of Assimilable Organic Carbon

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Introduction

Efficient operation of drinking water distribution system, home treatment devices, and reuse water lines is hindered by regrowth. Regrowth is the growth of microorganisms in a treated water. Regrowth in water distribution system, for example, has demanded a higher chlorine dose and thus has enhanced trihalomethane (THM) formation. Regrowth can occur only when compounds necessary or conditions stimulating it are prevalent. Therefore assessing the regrowth potential, and thus treating the water to remove regrowth supporting compounds is necessary.

Usually TOC cannot reflect the nature of organic compounds with respect to the ability to support bacterial growth or its biodegradability. Therefore new terminologies were introduced: Assimilable Organic Carbon (AOC) - the portion that can be converted to cell biomass and Biodegradable Dissolved Organic Carbon (BDOC) - the portion that can be mineralized by heterotrophic microorganisms.

Therefore a kind of bioassay is essential. There have been few bioassays proposed (Table 1). All of them either complicated requiring specialized measurements or when simple it does not reflect the actual situation. Use of a single strain or pure cultures has disadvantage: the interaction among other species is not represented. Usually HPC is used for assessing whether a particular distribution system suffers from regrowth. Therefore maximum HPC reached on PGY medium was tried as a measure of artificially added easily available organic carbon despite the facts that not all heterotrophic bacteria can be recovered and autotrophic bacteria are not recovered.

Table 1: Summary of AOC measurement methods

Method	Source of Inoculum	Incubation Temp, Time	Parameter Measured
Van der Kooij	Pure Cultures	15°C, 20days	cfu/mL
Kemmy	Mixture of 4 species	20°C, 7days	cfu/mL
USEPA (Coliform Growth Response Test)	Three coliform organisms	20°C, 5 days	cfu/mL
Werner	Sample	20°C, 2.5 - 5 days	cfu/mL
Jago - Stanfield	Raw water of Treatment or distribution system	20°C, Until max. ATP reached	ATP
Billen - Servais	Treatment Plant	20°C, 10-30 days	Bacterial Number, Size
Our Method	Sample	20°C, 5-10 days	cfu/mL

Materials and Methods

Test tubes and glass wares were prepared as follows; 1. dipping in detergent overnight, 2. washing with tap water followed by RO grade water, 3. dipping in dilute acid overnight, 3. washing 3 - 4 times each with tap, RO grade, and then Milli-Q water in sequence, and 4. finally heating overnight at 200°C.

Milli-Q water, and required organic or inorganic nutrients (Table 2) were added to test tubes and then autoclaved. After cooling down to room temperature, 1 mL of inoculum prepared by diluting 2µm filtered sample to get around 10^3 - 10^4 cfu/mL was added. Test tubes were placed perpendicularly on an inclined (around 45°C) axially rotating (about 50 rpm) disc incubated at 20°C for several days. Samples were taken using sterilized syringe and needle for HPC (Sakurai PGY medium, 20°C, 7days). Sampling

Table 2 : Nutrient concentration due to addition

Compound	Yield Exp. (µg/L)	Phosphorus Exp. (µg/L)
KNO ₃	305.8	505.5
KH ₂ PO ₄	39.9	66.0
Na ₂ SO ₄	13.6	22.5
CaCl ₂ ·2H ₂ O	5.6	9.2
MgCl ₂ ·6H ₂ O	12.6	20.8
FeCl ₃ ·6H ₂ O	7.4	12.2
CoCl ₂ ·6H ₂ O	0.15	0.26
CuCl ₂ ·2H ₂ O	0.20	0.33
MnSO ₄ ·5H ₂ O	8.39	13.86
ZnCl ₂	0.08	0.13
(NH ₄) ₆ Mo ₇ O ₂₄ ·4H ₂ O	0.04	0.07

frequency was higher for first few days, and lower in the later period.

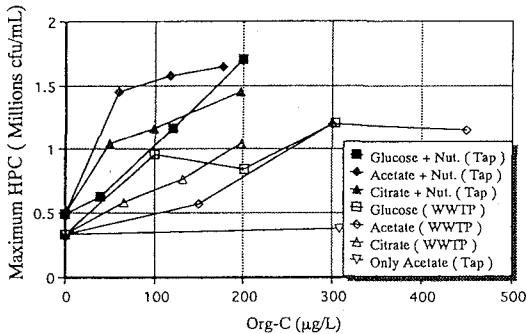


Fig.1: Yield Curves (Yield Exp.)

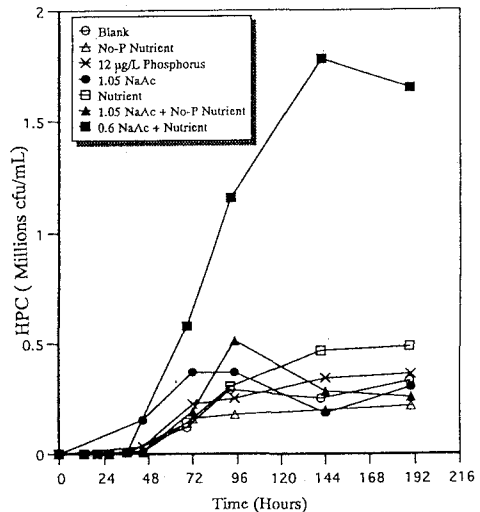


Fig.2: Comparison of growth curve for different conditions (Phosphorus Exp.)

Results and Discussion

Fig.1 is the result of two different yield experiments performed with; 1. wastewater treatment plant effluent (WWTP), and 2. tap water (Tap) inoculum. For WWTP case, only org.-C was added but for Tap case, except for 1.05 NaAc (1.05 mg/L acetate, i.e. 307 μg-C/L), nutrient salt solution was added. For WWTP case, maximum HPC seems linear for org.-C less than about 300 μg/L and it does not increase further with the org.-C addition. Addition of other nutrients for Tap case has increased the yield more than the WWTP while linearity with added org.-C remains valid within the range tested.

Linearity of maximum HPC with org.-C ($> 70 \mu\text{g/L}$ and $< 300 \mu\text{g/L}$) added indicates the possibility of using it as a measure of AOC. However checking this method for org.-C less than $70 \mu\text{g/L}$ is not possible until preparation of blank that has very small amount (near to zero) of easily available org.-C.

The results that HPC does not increase further with org.-C addition indicated the possibility that though org.-C is necessary for bacterial growth in a water having low org.-C concentration, other nutrients could as well control growth.

As a first trial (Phosphorus Experiment), effect of phosphorus presence was tested when tap water inoculum was used (Fig.2). While all cases (including only 1.05 NaAc added case and 1.05 NaAc + No-P nutrient added case) had their maximum below 0.5×10^5 cfu/mL, the complete nutrient salt solution added with 0.6 NaAc (175 μg - C/L) case of yield experiment had its maximum about four times. This clearly shows the possibility that phosphorus might play a major role in regrowth of bacteria. But the level at which it has to be maintained in order to control regrowth has yet to be investigated.

Conclusion

1. HPC can be used as a parameter indicating the amount of easily available org.-C (i.e. AOC indirectly) for org.-C greater than about $70 \mu\text{g/L}$.
2. Phosphorus might play a major role in regrowth of bacteria.

References

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2. Van der Kooij, D. (1982), Jour. AWWA, 74 (10) : 540-545