E.A. Anderson Engineering, Inc.

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PROJECT REPORT PR-2007-1: PRELIMINARY REVIEW OF THE EFFECTS OF pH AND TDS ON BACTERIA, VIRUSES, AND SPORES IN WATER

CONTENTS

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SUMMARY

Bacteria, Viruses, and Spores have critical portions of their structure made up from polymers of various acids, especially the 20 fundamental amino acids of protein chemistry, or the analogous nucleotide bases in RNA and DNA.

These biological species all rely upon a stable evironment of pH and salinity for their healthy existance. Once the pH of a water gets above pH 9.6, it is statistically highly improbable that any organism/spore/virus will have a peptide chain without at least some of the bonds being at sites which will have hydrolyzed. Increasing the pH to 9.7 virtually guarantees this effect, and it is common practice in sterilizing fermentation vessels to use a cleaning solution at pH 10 to ensure the removal of protein residues from the surfaces being cleaned.

The second aspect of interest is the role of dissolved solids or TDS. Dissolved solids are now ionic species, and can affect the salinity of the water. Where this appears to affect the biological activity of spores and cells is by denaturing various proteins (enzymes) required for reproduction, rendering the water biostatic. Therefore, high TDS waters should be biostatic to animal pathogens. The exact value varies by pathogen species.

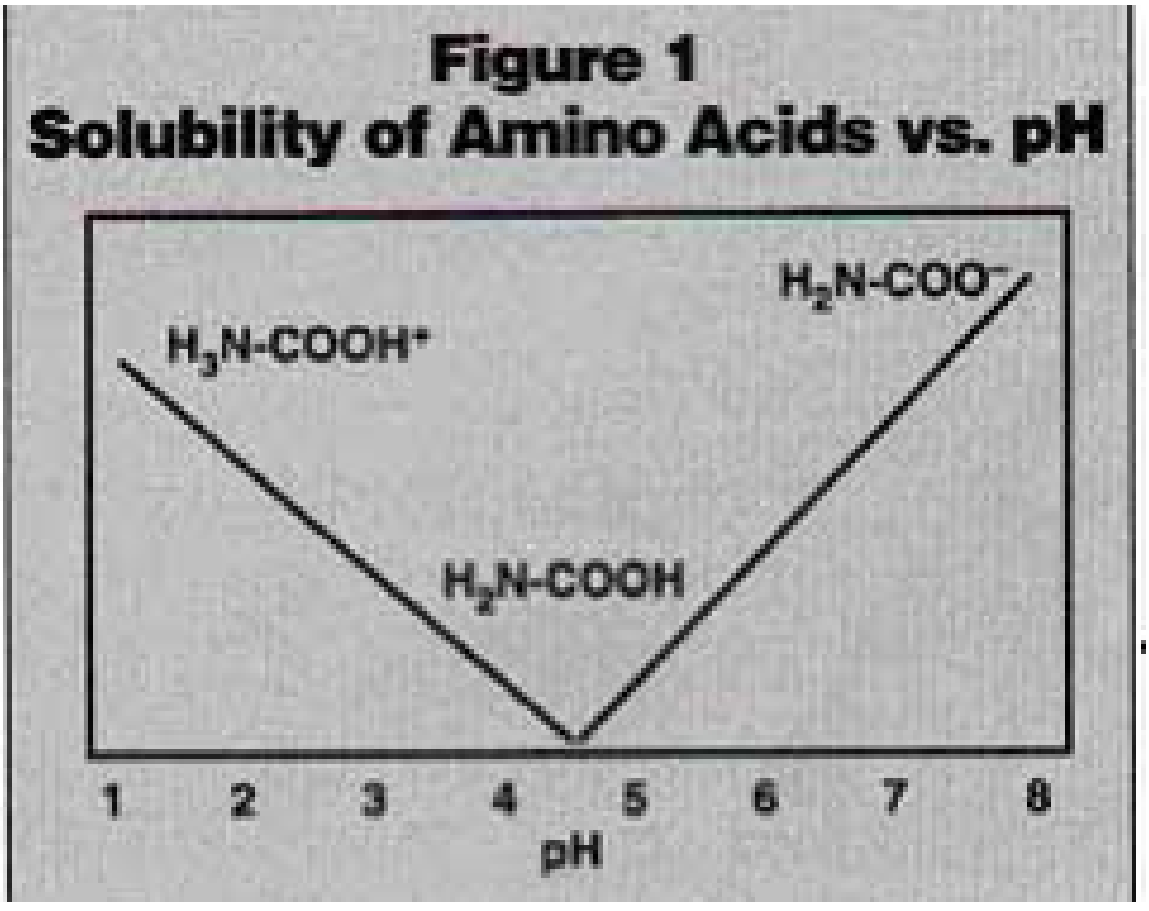
When the pH is greater than 9.7 and TDS values are over 40,000 ppm, biological activity would be expected to be blocked. Some especially hardy species may survive in a dormant state, and could be brought back to an active state when samples are withdrawn and diluted for laboratory analysis.

1. INTRODUCTION

Bacterial cell walls and viruses and fungal spores are polypeptide and or polynucleotide chains – proteins for the most part, with some other carbon – nitrogen bonds involved. The nucleotides have some analogous chemical bonding to the peptide chains, and conclusions about stability with regard to pH should be in line with the protein chemistry. In any case, RNA and DNA replicate by means of various enzymes, which are protein based molecules. Destroy or denature the enzymes, and the RNA and DNA molecules cannot replicate.

Polypeptide = polymer of amino acids.

General Hydrolysis of all polypeptides is known to occur at pH conditions greater than 10. This information has been collected from a variety of websites showing lecture notes and background information for introductory biochemistry classes. Professional research databases are also cited. Given the amount of time that has passed since the initial research was performed, citations tend to be from secondary sources.



General solubility of amino acids (typical ammonium ion pK_a ~ 10)

Since there are small variations in the specific pK_a values of amino and carboxylic acid groups in amino acids, the exact pH at which the predominant species is soluble varies somewhat. The pK_a values for the common amino acids are given in the tables in the Appendix.

Titration curves for the 20 fundamental amino acids show pK_a values of pH ~ 10 for the second acid group on each peptide. A good quick source of these curves is at the University of Virginia:

http://cti.itc.virginia.edu/~cmg/Demo/markPka/markPkaApplet.html

Another good source of this data is from the University of Arizona:
Database of the 20 Standard Amino Acids of Proteins:

http://www.biology.arizona.edu/biochemistry/problem_sets/aa/aa.html

This site corresponds to values contained in the PPD Database, the international clearing house for data for the 20 amino acids.

Another phenomena common to proteins is denaturing – the protein loses its characteristic shape – for example, an enzyme will not have any active sites while denatured. Denaturing due to exposure to high pH is reversible up until hydrolysis occurs. Thermal denaturing includes things like cooking of egg whites, and is not reversible. The denaturing environment can be due to soluble salts, such that a high TDS water could denature the various biota without hydrolyzing the structures.

http://en.wikipedia.org/wiki/Denaturation_(biochemistry)

Denatured cell walls and peptide chains would render the biota inactive so long as they remain in the inhospitable environment. Removing the biota sample, then diluting the solution to reduce the cell count will also result in re-activating these cells. Spores, enzymes, and viruses behave in similar manners.

The specific conditions will vary for each species. It is possible that the wastewater treatment people have come up with a statistical basis for disinfecting their processes – more research needs to be done before that possibility can be confirmed.

One discussion forum for wastewater operators has an interesting discussion of the effects of brines on activated sludge:
http://www.wef.org/technicaldiscussions/Topic514-14-1.aspx#bm1887

Going back to the hydrolysis effect, there are the 20 fundamental amino acids contained in all proteins and DNA, and other natural biochemicals. Referring to the pK_a values given in the tables in the Appendix, these peptides have the 2nd pK_a (aka pK_2) values (the peptides have two functional groups each) in the range from 8.8 to 12, with most of the acids clustered at 9.6 to 9.7 Half of the peptides (10 of 20) are reactive below 9.4 pH If the pH is raised to 9.7, 17 of the 20 peptides will hydrolyze.

The reasoning behind the tradition of raising the pH of wastewater to ~10 then neutralizing it is that if 17/20 = 85% of the peptides in the polymeric chains of the organisms are hydrolyzed, none of the organisms can survive.

At a pH of 9.4, 50% of the peptides will hydrolyze, ensuring that most organisms will not survive. Raising the pH to 9.6 gets 60% of the peptides, and pH 9.62 will hydrolyze 70% of the peptides.

Statistically, it is highly improbable that any organism/spore/virus will have a peptide chain without at least some of the bonds being at sites which will hydrolyze at pH 9.6

APPENDIX

PPD v1.0—an integrated, web-accessible database of experimentally determined protein pK_a values

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The PPD data was sourced from the primary literature and contains in excess of 1400 entries. The database contains pK_a values for amino acid side-chains, as well as the N and C termini, over 75% of which focus on Glutamate, Lysine, Histidine and Aspartate. These four residues are all key ionisable residues, and therefore the apparent bias is not driven by our selection, but by the available experimental data. Very little data is currently available for Arginine: its pK_a value (~12) essentially precludes measurement by titration as proteins will denature at high basic pH.

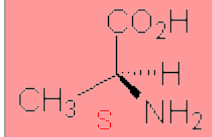
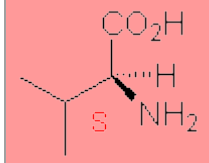
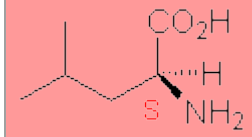
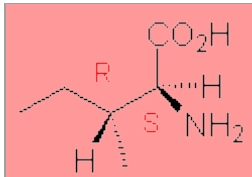
1.1.1 Structure and Properties of Amino Acids

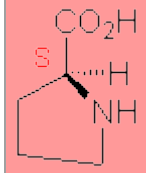
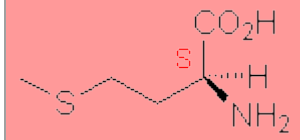
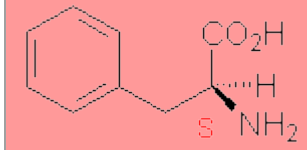
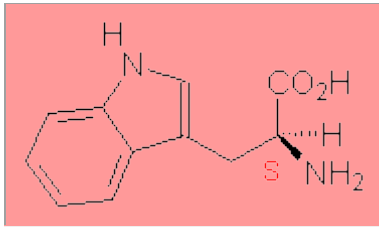
Mother Nature builds proteins from a set of "blocks" consisting of 20 amino acids, shown in the Table below. They all have the same α-amino acid structure, with the same arrangement of ligands around a stereogenic center (except glycine, which is achiral).

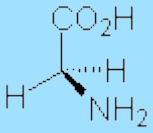
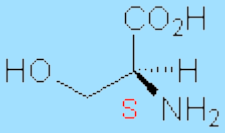
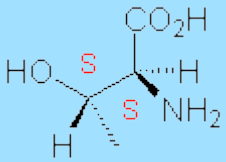
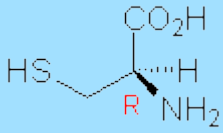
The line below each structure contains the three- and one-letter abbreviations widely used for the acid, the approximate natural abundance in typical proteins, and the pK_a values.

- Although amino acids under physiological conditions exist as zwitterions, in which the carboxyl proton is transferred to the amino group, they are shown here as neutrals.

The background color groups the acids by side-chain category as: (1) nonpolar or hydrophobic (red); (2) polar, uncharged (blue); (3) negatively charged (green); and (4) positively charged (lavender).

Alanine	Valine	Leucine	Isoleucine
			
Ala, A; 7.8%; 2.35, 9.87	Val, V; 6.6%; 2.29, 9.74	Leu, L; 9.1%; 2.33, 9.74	Ile, I; 5.3%; 2.32, 9.76

Proline	Methionine	Phenylalanine	Tryptophan
			
Pro, P; 5.2%; 1.95, 10.64	Met, M; 2.2%; 2.13, 9.28	Phe, F; 3.9%; 2.16, 9.18	Trp, W; 1.4%; 2.43, 9.44

Glycine	Serine	Threonine	Cysteine
			

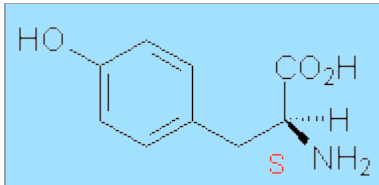
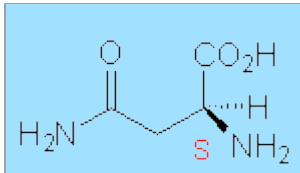
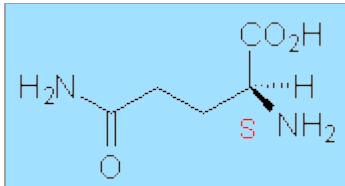
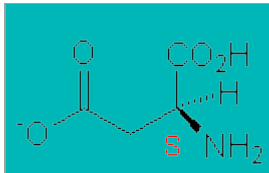
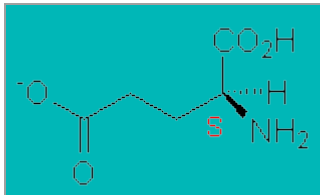
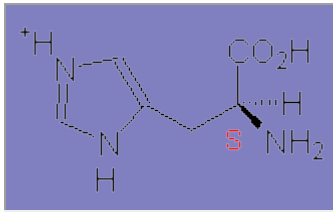
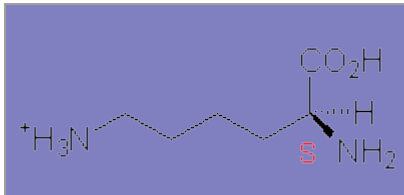
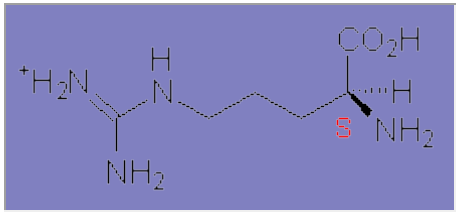
Gly, G; 7.2%; 2.35, 9.78	Ser, S; 6.8%; 2.19, 9.21	Thr, T; 5.9%; 2.09, 9.11	Cys, C; ?; 1.92, 8.35, 10.46
Tyrosine	Asparagine	Glutamine	Aspartic Acid
			
Tyr, Y; 3,2%; 2.20, 9.11, 10.13	Asn, N; 4.3%; 2.1, 8.84	Gln, Q; 4.3%; 2.17, 9.13	Asp, D; 5.3%; 1.99, 3.90, 9.90
Glutamic Acid	Histidine	Lysine	Arginine
			
Glu, E; 6.3%; 2.10, 4.07, 9.47	His, H; 2.3%; 1.80, 6.04, 9.33	Lys, K; 5.9%; 2.16, 9.18, 10.79	Arg, R; 5.1%; 1.82, 8.99, 12.48

table 5–1

Properties and Conventions Associated with the Standard Amino Acids

Amino acid	Abbreviated names		M_r	pK_a values: pK_1 (—COOH)	pK_2 ($—NH_3^+$)	pK_R (R group)	pI	Hydropathy index*	Occurrence in proteins (%)†
Nonpolar, aliphatic R groups									
Glycine	Gly	G	75	2.34	9.60		5.97	−0.4	7.2
Alanine	Ala	A	89	2.34	9.69		6.01	1.8	7.8
Valine	Val	V	117	2.32	9.62		5.97	4.2	6.6
Leucine	Leu	L	131	2.36	9.60		5.98	3.8	9.1
Isoleucine	Ile	I	131	2.36	9.68		6.02	4.5	5.3
Methionine	Met	M	149	2.28	9.21		5.74	1.9	2.3
Aromatic R groups									
Phenylalanine	Phe	F	165	1.83	9.13		5.48	2.8	3.9
Tyrosine	Tyr	Y	181	2.20	9.11	10.07	5.66	−1.3	3.2
Tryptophan	Trp	W	204	2.38	9.39		5.89	−0.9	1.4
Polar, uncharged R groups									
Serine	Ser	S	105	2.21	9.15		5.68	−0.8	6.8
Proline	Pro	P	115	1.99	10.96		6.48	1.6	5.2
Threonine	Thr	T	119	2.11	9.62		5.87	−0.7	5.9
Cysteine	Cys	C	121	1.96	10.28	8.18	5.07	2.5	1.9
Asparagine	Asn	N	132	2.02	8.80		5.41	−3.5	4.3
Glutamine	Gln	Q	146	2.17	9.13		5.65	−3.5	4.2
Positively charged R groups									
Lysine	Lys	K	146	2.18	8.95	10.53	9.74	−3.9	5.9
Histidine	His	H	155	1.82	9.17	6.00	7.59	−3.2	2.3
Arginine	Arg	R	174	2.17	9.04	12.48	10.76	−4.5	5.1
Negatively charged R groups									
Aspartate	Asp	D	133	1.88	9.60	3.65	2.77	−3.5	5.3
Glutamate	Glu	E	147	2.19	9.67	4.25	3.22	−3.5	6.3

*A scale combining hydrophobicity and hydrophilicity of R groups; it can be used to measure the tendency of an amino acid to seek an aqueous environment (− values) or a hydrophobic environment (+ values). See Chapter 12. From Kyte, J. & Doolittle, R.F. (1982) *J. Mol. Biol.* **157,** 105 – 132.

†Average occurrence in over 1150 proteins. From Doolittle, R.F. (1989) Redundancies in protein sequences. In *Prediction of Protein Structure and the Principles of Protein Conformation* (Fasman, G.D., ed) Plenum Press, NY, pp. 599–623.

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The Journal: **Water Research**

California Coastal Commission Website: http://www.coastal.ca.gov/desalrpt/dkeyfact.html