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Karen Rippe
Kennesaw State University, kduda1@kennesaw.edu

Brian Condon
The United States Department of Agriculture, Southern Research Center, New Orleans, LA

Kevin Hosterman
Kennesaw State University

Erica Williams
Kennesaw State University

Michelle Garner
Kennesaw State University

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A Survey of Enzymatic Activity in Commercially Available Pool and Spa Products

Karen Rippe, Brian Condon, Kevin Hosterman, Erica Williams, and Michelle Garner

***Key Words**:* swimming pool maintenance, swimming pools, water clarity, water decontamination, water filtration

Traditionally, chlorine or bromine products have been used to keep pool water free from bacteria, algae, and other contaminants such as oils and nitrogen-based materials. Chlorine and bromine have been safe and effective in water-purification applications for many years. If handled improperly, however, these chemicals could pose environmental or human safety problems (National Swimming Pool Foundation, 2006). For these reasons, alternatives or supplements to the use of chlorine and bromine would be beneficial. One alternative is to use enzymes to break down pool contaminants to aid filtering and reduce film or scum formation.

One such enzyme is a lipase (Aehle, 2004). Lipases are enzymes that break down lipid molecules into simple fatty acids and glycerol. They are naturally occurring molecules produced by plants, animals, bacteria, and mold. Lipase enzymes are capable of working in a water and oil emulsion and therefore act at the oil–water interface where chlorine and bromine products often cannot reach or react slowly. Enzymes are catalysts, and, as such, one enzyme molecule can break down many lipid molecules. This feature allows enzymes to be effective at concentrations well below that required for the chemical interaction of chlorine with the lipids, because chlorine reacts with lipids in a 1:1 ratio. Figure 1 shows the chemical structure of a typical lipid found in lotions, suntan oils, and many hair products. The arrows indicate where the lipase cleaves the lipid, producing glycerol and a fatty acid. Once the lipid is broken down, the detergents found in the pool products can then solubilize the smaller molecules, allowing them to be filtered out of the pool. Because triglycerides (a type of lipid) are involved in the practical maintenance issues of scum lines, surface films, and filter clogs, the lipase could be especially beneficial in preventing those problems.

One factor limiting the use of enzymes is that humans have been known to develop allergies to them with extended exposure. A second downside is that

Rippe, Hosterman, Williams, and Garner are with the Dept. of Chemistry and Biochemistry, Kennesaw State University, Kennesaw, GA 30144. Condon is with the United States Department of Agriculture, Southern Research Center, New Orleans, LA 70124.

enzymes are proteins and therefore can be inactivated by heat, UV light, chlorine/bromine, detergents, and other chemicals found in pool water. The rate at which enzymes are inactivated can be highly variable depending on the stability of the specific enzyme and how it is incorporated into the pool product.

In this study we used a colorimetric assay to measure the activity of the enzymes in commercially available pool products (Frodyma, 2005). Because enzymes can work at low concentrations, it is impractical to directly measure the products of the lipase reaction with lipids. Colorimetric assays rely on the use of a molecule attached to the substrate for detection. In this case paranitrophenyl butyrate, or pNP-butyrate, was used as the substrate for the lipase to degrade. When a lipase cleaves the pNP-butyrate into p-nitrophenol and butyric acid, the p-nitrophenol turns a yellow color that can be monitored by a spectrophotometer at 405 nm. Figure 2 shows the chemical reaction catalyzed in the assay experiment.

With dozens of these products currently on the market, there has been no published uniform test to determine what enzyme activity is present as purchased or that the products will be active and act effectively when diluted into the pool water as directed. Furthermore, because chlorine and bromine also perform the

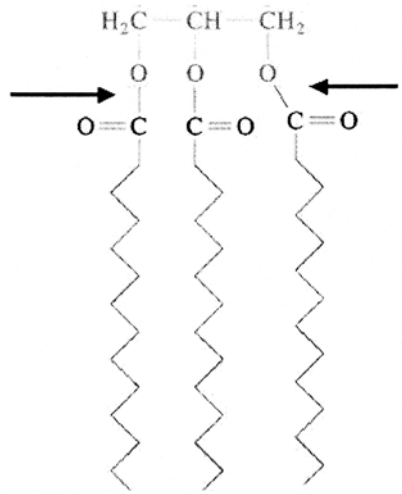

Figure 1 — The structure of a lipid. The arrows indicate where the lipase cleaves the lipid. The functional group cleaved is mimicked in pNP-butyrate.

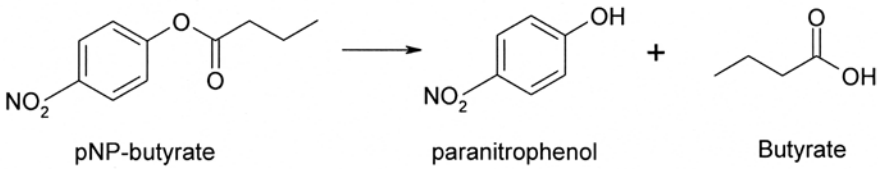

Figure 2 — The chemical reaction catalyzed by lipases. The paranitrophenol absorbs light at 405 nm, allowing a measurement of lipase activity based on product formation.

same function of degrading lipids in pool water, there have been no published tests to determine how effective the enzymes are relative to the activity of chlorine and bromine. This study focused on determining the level of enzyme activity in these pool-water treatment products and how effectively that activity is performed in diluted water.

Materials and Methods

All colorimetric assays were performed in Falcon 24-well microplates using pNP-butyrate as the substrate and detected using a Packard SpectraCount microplate reader. Dilutions were prepared in either buffer (1.15 g dibasic sodium phosphate, 0.200 g monobasic potassium phosphate, 8.00 g sodium chloride, 0.200 g potassium chloride, and 2.00 ml Triton-X 100 per liter at pH 7.2) or water obtained from a local swimming pool as the test required. All pool water was determined to be between pH 7.1 and 7.2 with chlorine levels between 1–2 ppm. These readings were obtained using Taylor's "Service Complete" pool kit to ensure comparable results with those obtained by pool maintenance staff. Pool products were purchased from local pool-product vendors in a manner consistent with that of the average consumer.

A substrate solution of 100 mM pNP-butyrate (Sigma Chemicals) was prepared by mixing 20 ml of acetonitrile with 250 µl of pNP-butyrate and stored at –20 °C for up to 2 weeks. Immediately before running each assay, this substrate was further diluted 1:50 in buffer. Each of the 24 wells contained a total volume of 2.000 ml: 1 ml buffer or pool water, 0.500 ml pNP-butyrate substrate solution, and 0.500 ml of the pool product diluted in buffer or pool water as the test required. Each sample was performed in duplicate, and the results averaged. The assay was run at room temperature (20 °C) unless otherwise noted. Absorbance readings were taken at 405 nm every 15 min for 90–120 min. For samples using inactivated pool product, the product was boiled for 20 min before diluting in pool water. In these assays, the enzyme activity is proportional to the rate of increase of absorbance at 405 nm, which is given by the early reaction slope of the absorbance-versus-time plot for the assay. In this manner, relative enzyme activities are determined.

Results and Discussion

The first question was how much enzymatic activity, if any, the products contained as packaged. Figure 3 shows the relative enzyme activity of several pool-water treatment products tested as formulated. The results show that of the 12 products tested, 6 contained some lipase activity. Of the six products that did contain lipase activity, however, there was a wide variety of activity levels.

In an attempt to evaluate enzyme activity at the recommended dilution level for in-use application, the products required dilution at approximately 13,000:1. The assay was not sensitive enough to detect activity at that dilution level, however, so a series of pool-water treatment product dilutions was examined to determine an effective dilution of the products for the remainder of our studies (Figure 4). The overall enzyme activities in the products were somewhat low, and the recommended in-use dilutions were quite large. This might lead one to suspect a priori that the

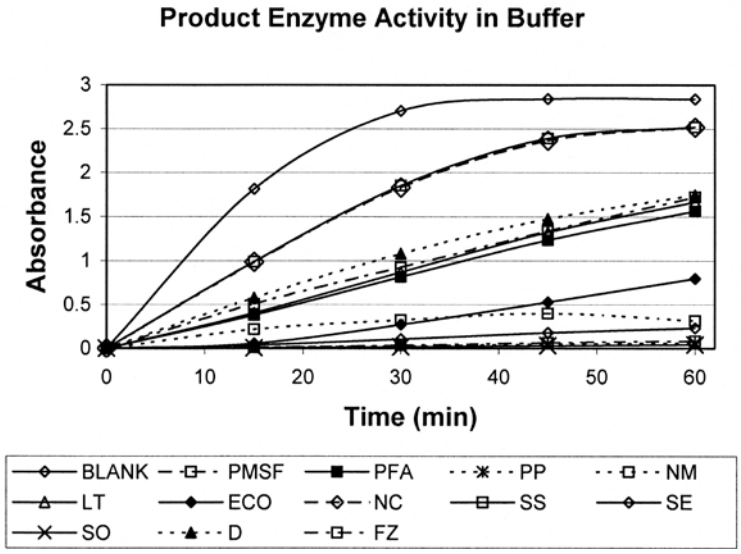

Figure 3 — Activity of formulation-strength pool products. To ensure maximum lipase detection, no dilutions of the products were performed for this assay. A buffer blank was used to zero all the readings. PMSF = Pool Magic Spring and Fall; PFA = Pool First Aid; PP = Pool Perfect; NM = Natures Magic; LT = Leisure Time; ECO = Eco One Spa Treatment; NC = Natural Clear; SS = Spa Scum Digester; SE = Spa Essentials; SO = Scum Out; D = Dissolve; FZ = Filter Zyme.

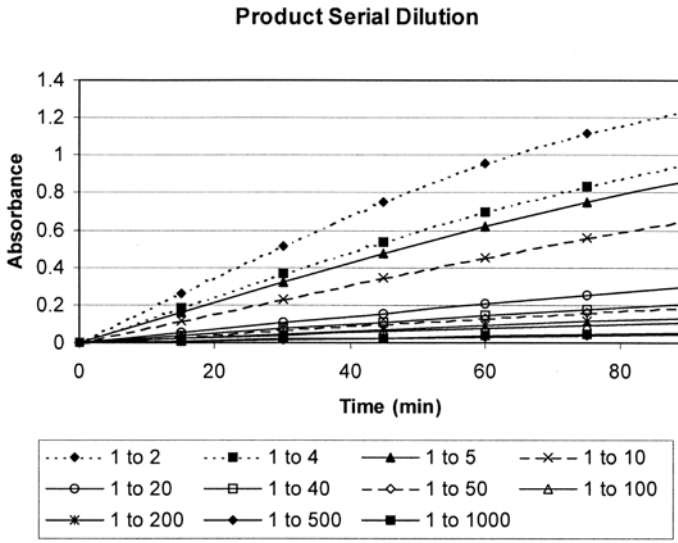

Figure 4 — Serial dilutions of the LT pool product to determine study dilution level. Products were diluted as indicated in the figure. Buffer was used for these dilutions. The same assay was performed for all products in the study.

lipase activity might not be effective at lipid degradation at in-use conditions. Nonetheless, in-use application allows for a very extended period of time for the enzyme to be effective, whereas our experimental assay required a 150-min run time for practicality. Therefore, we determined that using a 1:20 dilution provided the best compromise among these factors while allowing detection of differences in activity level in a reasonable amount of time. This lower dilution factor would also ensure that the enzyme had sufficiently conducive reaction conditions to compete effectively against the degrading action of the chlorine.

To compare the lipid-degrading capacity delivered to the pool water by the lipase enzyme with this same activity produced by chlorine commonly found in pool water, a standard assay was designed and run for each pool-water treatment product. In this assay, each product was tested under several conditions. As a blank, the hydrolytic activity of buffer alone was measured. This allowed a measurement in the absence of both product and chlorine to provide a measure of autolysis, or hydrolysis simply caused by water and not as a result of the product or the chlorine. The second condition measured the hydrolytic activity of just pool water, to determine the hydrolytic activity of chlorine in the water once the autolysis contribution had been subtracted. The third condition measured the hydrolytic activity of pool-water treatment product diluted in pool water. Thus, the hydrolytic activity from just the pool water can be subtracted from this condition, providing a measure of the enzyme activity resulting from that pool-water treatment product and presumably the lipase contained therein. The fourth condition involved a measurement of a dilution in pool water of pool-water treatment product that had been previously boiled for 20 min to inactivate the lipase enzyme contained therein. This experiment determined any hydrolytic activity that occurred as a result of other chemicals in the pool-water treatment product. Finally, one pool product diluted in buffer was run as an internal control to ensure that the pool-water treatment products did not loose enzyme activity during the course of study.

Because most of these products are used in pools and spas that are operated at higher temperatures, we initially attempted to perform the assays at 85 °F (29.5 °C; Figure 5). Unfortunately, both the buffer and pool-water blanks obtained maximum activity in 75 min. This indicated that the substrate pNP-butyrate undergoes significant autolysis (normal degradation not resulting from chlorine or lipase) at higher temperatures. Because of this high autolysis rate, all other data reported in this study were collected at lower temperatures (68 °F/20 °C) to eliminate the autolysis.

Data collected for one pool-water treatment product using the assays described above at 68 °F (20 °C) is shown in Figure 6. All products were tested under identical conditions. The complete data set for all products is compiled in Table 1. Although six of these products displayed statistically significant enzymatic activity, the 1:20 dilution used in the assay is much more concentrated (by 6,500 to 65,000 times) than the directions called for in an actual pool setting. A 1:20 dilution would be the equivalent of 1 oz (29.5 ml) per 0.16 gal (590 ml). The suggested doses ranged from a 1:13,000 dilution to a 1:129,000 dilution. The highest enzyme activity in our study (Natures Magic) at a 1:20 dilution had a corresponding absorbance value of 2.433 (Table 1). If Natures Magic were used as directed, however, at a dilution of 1:13,000, the value resulting from the enzyme activity would be 0.0037 according to dilution calculations. This is not indicative of significant enzymatic activity from the pool-water treatment product at the recommended dosage levels. In comparison,

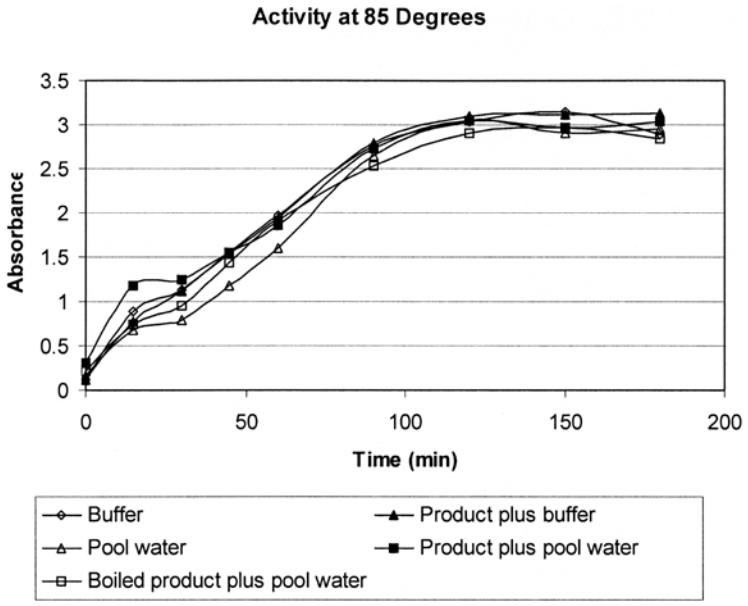

Figure 5 — Results of the standard assay performed at 85 °F (29.5 °C). The high activity of the buffer and pool-water blanks indicates that the substrate is undergoing autolysis.

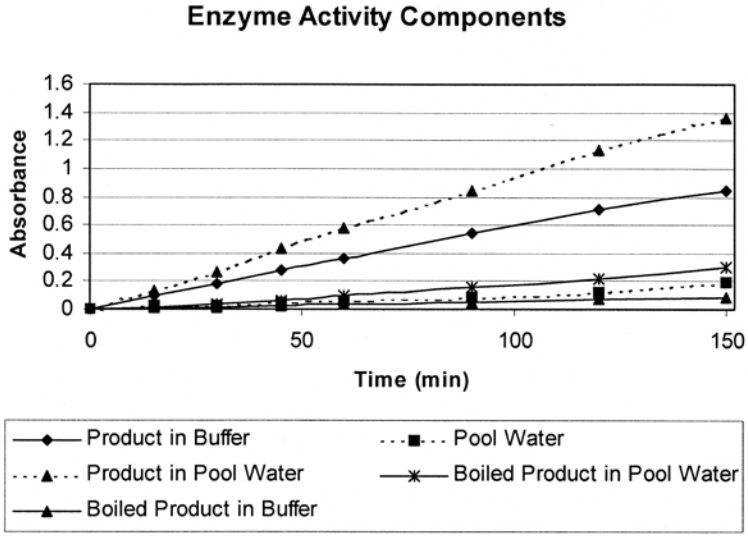

Figure 6 — Activity levels under various dilution conditions used to differentiate the source of the lipid degradation. All products were diluted 1:20 in either buffer or pool water as indicated in the figure, and the assay was performed at 68 °F (20 °C).

Table 1 Factors Contributing to Lipid Degradation

Pool product	Total activity	Detergent activity	Chlorine activity	Enzyme activity
Natures Magic	3.000	0.382	0.185	2.433
Leisure Time	0.874	0.175	0.203	0.496
Pool Zyme	0.395	0.000	0.404	0.000
Pool Perfect	0.495	0.026	0.277	0.192
Filter Zymea	0.145	0.000	0.078	0.067
Spa Scum Digester	1.375	0.103	0.207	1.065
Pool First Aid	0.267	0.000	0.272	0.000
Spa Essentials	0.242	0.083	0.177	0.000
Natural Clear	1.319	0.154	0.173	0.992
Scum Out	0.055	0.000	0.158	0.000
Dissolve	1.707	0.285	0.213	1.209
Pool Magic	0.195	0.015	0.203	0.000

Note. Values were calculated as follows using data from the 1:20 product-dilution studies (Figure 6): Total activity = activity in the product in pool-water sample; chlorine activity = activity in pool-water sample; enzyme activity = total activity – boiled product in pool-water sample; detergent activity = total activity – chlorine activity – enzyme activity.

[a]The chlorine activity of this assay is statistically different from all other assays run, so no conclusions about the product Filter Zyme can be determined based on this assay.

the assays containing only pool water displayed much higher activity than this, having an average activity of 0.213. Because the concentration of chlorine in the pool water is held constant within a small range, this activity level is representative of actual pool use. Therefore, the pool water itself contributed to significantly more lipid degradation than the lipase activity contained in the pool-water treatment product. Although the products do contain lipase activity, based on these results it does not appear that the pool products would lead to significant enzyme-derived hydrolytic activity at the directed dosage. It might still be possible, however, that in a highly used pool with many scum-line and filter problems or in an application operating under significantly different in-use conditions the ability of the enzymes to contribute to lipid degradation might provide some benefit beyond normal chlorine use. This was not tested in this study, however.

Acknowledgments

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