Doesn't Play Well with Others- The Chemistry of the Autoclave

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While Luria-Bertani broth (LB) has long been the fuel that powered Molecular Biology and Biochemistry, there is an increasing movement towards more specialized and complex bacterial media formulations such as Terrific Broth (http://openwetware.org/wiki/Terrific_Broth) (TB), Plasmid DNA Media (https://bitesizebio.com/articles/pimp-your-plasmid-growth-medium/) (PDMR), and Autoinduction Media (http://openwetware.org/wiki/Lidstrom:Autoinduction_Media) (ZYP-5052). These media formulations optimize *E. coli* cell growth and performance utilizing specialized carbon sources and phosphate buffering systems, and the preparation of these media always solicits the same question: Why can't I autoclave all the ingredients together? The answer: chemistry.

Sugars and Amino Acids

Sugars are often added to media to serve as an energy rich carbon source (glucose in PDMR), and sometimes to also elicit a particular metabolic response from the bacteria (glucose and lactose in ZYP-5052). The base for almost all media is amino acid-rich powders such as yeast extract and tryptone. There isn't any apparent incompatibility with sugars and amino acids, and there isn't – at room temperature. In the autoclave, however, sugars and amino acids fall victim to the Maillard reaction (http://en.wikipedia.org/wiki/Maillard_reaction). In this reaction, the amino group of an amino acid reacts with a carbonyl group of a sugar, fusing the two molecules together. This is a common reaction in our kitchens, making bread crusts brown, and changing the flavors of foods as we cook them.

The problem with this occurring in our media is two fold: first you are exchanging valuable molecules for ones that the bacteria might not be able to utilize, and second, these new molecules tend to be unstable and break down into compounds that may actually inhibit bacterial growth. These types of

compounds are formed frequently enough that back in the day there were papers written describing the detrimental effects of autoclaving on bacterial media. The extent with which this reaction occurs depends on many variables, including the types of sugars that are present, the length of time that the media is autoclaved, and the types of salts that are present. Therefore, sugars should be autoclaved separately from amino-acid (and protein) containing solutions, if at all.

Sugars and… Themselves

Caramelization (http://en.wikipedia.org/wiki/Caramelization), also tasty chemistry that commonly occurs in the kitchen, is the heat-induced breakdown of sugars. Although caramelization isn't as chemically defined as the Maillard reaction, it also produces browning and can generate some compounds inhibitory to bacterial growth. The reaction depends on temperature, time (at elevated temperatures), the type of sugar, and pH. Although caramelization often doesn't occur during autoclaving if the autoclave is calibrated correctly and the sugar solution is retrieved promptly at the end of the run, if the conditions are right, then it can occur. For this reason many researchers choose to sterilize sugar solutions by filtration.

Phosphates and Metals

The growth of *E. coli* cultures is inhibited at low pH, and growing cultures release acids into the media. Therefore including a buffering system to the media will increase the maximum cell density attainable, and a mixture of mono- and di-basic phosphates is a cheap buffer system to keep the pH of a culture near neutral. In addition, some of the phosphate can be utilized by the growing culture as a nutrient. The only problem with phosphate is that it tends to precipitate ionic metals in a temperature and pH dependant manner. Even media that hasn't had metals specifically added to it still likely contains trace amounts that were brought in from the yeast extract or tryptone.

If you autoclave the media with phosphate added to it, a precipitate will often form that does not form if the two components are autoclaved separately and then mixed at room temperature. This isn't necessarily intuitive, since the solubility of many of the salts that we work with are enhanced at higher temperatures, however any salt that undergoes exothermic dissolution will be prone to precipitation at higher temperatures. To complicate matters, any significant precipitation of phosphate from the media will likely change the pH of the media, since the cation will selectively pull down one ionic form of the phosphate. Depending on the cations that precipitated with the phosphate and the resulting pH change of the media, the precipitate may not go back into solution once the media cools. (It would with a pH adjustment, but that generally cannot be done while maintaining sterility). Consequently, phosphate solutions are autoclaved separately, and aren't added to the other media components until the temperatures are below 40°C or so.

Although separating some of the components of these specialized media adds a small amount of time to their preparation, doing so increases the consistency of their performance. If you have any other hints or tips to add, let us know in the comments!

Written by Jode Plank (https://bitesizebio.com/profile/jode-plank/)

12 Comments

Sunny Yoda on May 29, 2019 at 5:21 pm

Hi all,

I am having trouble growing S. cerevisiae, was wondering if anyone can help.
So when I innoculate my cells in SC media in which glucose was added and then autoclaved the cells grows fine, but when I innoculate my cells in SC media with filtered glucose, the cells do not grow at all. I have done this multiple times and still get the same result. Can anyone help me understand what is going on?

I am innoculating 100ml of media with 50ul of 0.6OD cells.

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Mercy on June 22, 2016 at 9:56 pm

I am now seeing this post and its very helpful.For my media preparations, i have my sugars(glucose, xylose, arabinose) made separately and my yeast extract and the phosphate together and autoclave separated at 121 degrees for 15 mins and never experienced any precipitation, caramelization or maillard reaction.my microorganism grows beautifully.now my concern is the YE/phosphate mixture.since i am not physically seeing the precipitates how can i verify there isn't any formation?

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Rehan on December 30, 2015 at 10:08 am

Hi.
I am the student of MSc in plant tissue culture laboratory. We have faced a problem while preparing our tissue culture media. When we autoclave the media then insoluble precipitations are being formed. Is anyone have any solution regarding this problem.

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Kurt on February 24, 2011 at 6:16 am

There are many ready mixed rich media which contain extra carbohydrates (usually glucose), if you are careful to really keep the autoclaving time to the minimum it usually works. But just a few more minutes of autoclaving and it turns dark, glucose seems less dangerous than e.g. lactose, which always has to be added separately.

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Jode Plank on February 24, 2011 at 3:13 am

Historically, the separation of the sugars from the base during autoclaving was driven by necessity – the organism of interest (E. coli, plants, etc) would show variable growth kinetics that the researchers had to fix, and separation of the sugars during heat sterilization was often the fix. Perhaps Lactobacillus simply isn't inhibited by the by-products of the Maillard reaction, so the pioneers of the field never needed to separate the glucose from the peptone.

Does the media darken during autoclaving?

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Christopher Dieni on February 24, 2011 at 2:20 am

Interesting! Then why is it okay to autoclave media containing sugars and peptone? For example, on almost a daily basis, I autoclave MRS media (named after its inventors, de Man, Rogosa and Sharpe) for Lactobacillus growth. This media contains 1% peptone (proteolytic digests of animal milk or meat resulting in a variety of peptides) and 2% glucose, yet it's perfectly safe to autoclave.

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Thecreativedna on May 23, 2012 at 2:09 am

Christopher- i am not sure but no wrong effect on MRS medium After autoclaving May be due to the pH of medium. Milliard reaction is favoured by high temperature moistur content and high pH i. e. alkaline conditions. As the pH of the MRS medium ranges in acidic range which is 6.2 it Will not result in damage of sugar present in medium..

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Solikhin on June 23, 2019 at 6:23 am

I am doing autoclaving for MRS medium. after that, the medium didn't change the color. The final color of the medium still yellow. What happens with my MRS medium? can you explain to me, thanks.

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Jode on February 23, 2011 at 5:50 pm

Chris – I have every reason to believe that the N-terminal amino group of a peptide would undergo the Maillard reaction. As for other compounds with amino groups, such as nucleotides, I don't know if they would react with sugars or not.

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Jode on February 23, 2011 at 5:35 pm

Sexcomb – You raise a good point. Kit protocols are almost always assuming that you use LB, and have recommended a culture volume based on that. If you are using an enhanced media, you should probably be using a smaller volume of culture to get the same bacterial pellet size. After all, the amount of DNA you can get from a kit is limited by the capacity of the column they give you.

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Christopher Dieni on February 23, 2011 at 5:26 pm

Jode, thanks so much for writing this. This has answered so many questions I had about why certain components just couldn't be autoclaved together! I just grumbled and followed the directions without being adventurous, but if I had, now I know what would've happened.
Just a chemistry question, more out of curiosity than anything else. Plenty of components of media have free amino groups. In fact, the peptides found in various formulations of peptides have N-terminal amino groups, just like an amino acid would. Why are these not undergoing the Maillard reaction as well?

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Sexcomb on February 23, 2011 at 5:04 pm

Be careful with TB. More bacterial cells generate more debris which can clog plasmid isolation kits. Be sure to try the new media in one or two minipreps to see if you can process the larger cell pellet.

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