ABE 304
Bioprocess Engineering Laboratory

BIOENCAPSULATION OF LIVING BACTERIA IN XANTHAN/GELLAN GUM BEADS

INTRODUCTION AND SCOPE

Bioencapsulation. Bioencapsulation refers to the trapping of a biological entity in a matrix material, such as a polymer gel or porous glass. The encapsulation of cells, viruses, or biomolecules (such as bacteria, phage, or enzymes) has important applications in food production, biotechnology and biomedicine. For example, probiotic bacteria can be encapsulated into an edible matrix such as gelatin, alginate, starch or gums (e.g. xanthan gum) to protect the bacteria during food formulation such that when ingested the "healthy" bacteria can incorporate into the host gut (Burgain et al. 2011). In other examples, bacterial biofilms can be encapsulated within inorganic matrix materials in a membrane bioreactor to prevent the detachment and loss of bacteria cells during operation of the reactor for water remediation (Jaroch et al. 2011).

One of the most common examples of bioencapsulation is the encapsulation or immobilization of enzymes. In the ABE department, research in incorporating proteins including enzymes into materials with nanometer or micron scale dimensions to make biosensors is performed (Rickus lab). The most well known commercial biosensor is a glucose test strips that measures glucose in the blood for people with diabetes. These test strips have an enzyme, immobilized or encapsulated on the test strip. Bioencapsulation generally occurs by embedding the cells, phage, or biomolecules into a porous gel or solid porous material, which may be organic or inorganic in nature depending on the application. Because typically the encapsulated entity must remain alive and/or functional, the assembly of the matrix must take place under chemical conditions that are compatible with biomacromolecules (i.e. physiological pH, temperature). In addition, often fluid and small molecule exchange between the encapsulated cell and the surrounding is necessary to keep a trapped cell alive.

Gums as Materials for Bioencapsulation. Gums are polysaccharides, or long polymer carbohydrates, that have widespread commercial application in food, pharmaceutical, and biotechnology applications. Gums occur in nature and have been adapted for commercial production as a raw ingredient (Table 1). Agar is a staple of microbiology and plant biology as a solid media for bacterial and plant cell growth. Alginate, derived from seaweed, is one of the most common materials investigated for the encapsulation of human cells prior transplantation to cure disease (e.g. type I diabetes). Gums such as xanthan, guar, and carrageenan are widely used in the food industry to influence the rheological properties of processed foods. These rheological properties correspond to consumer preferences around how the product feels in their mouth or on a spoon or knife.

Bioencapsulation of Probiotics for Delivery in Foods and Medical Products. Many types of foods such as yogurts, unfiltered beer, and fermented vegetables, contain live and/or dead microorganisms. With increasing knowledge of the importance of the human microbiome on health and disease, these foods are experiencing significant growth in popularity. New products that deliver natural or engineered microorganisms to the body as a medical therapy or for more general health benefit are also emerging. In order to provide a benefit, the cells must remain viable in the food and in the early stages of digestion. Bioencapsulation therefore is an important technology for probiotic delivery in foods and medicine. Because of their natural and food grade status and their ability (in some cases) to form gels, gums are an attractive material for encapsulation matrices. You have already learned about xanthan gum in your other labs. In this lab we will use a gellan / xanthan gum mixture to create gels in which we will bioencapsulate *E. coli*.

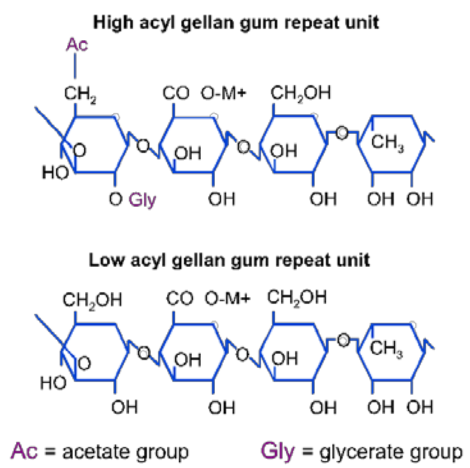
Table 1. Commercial gums organized by source. Source: ("KelcoGel gellan gum book" 2007)

Table 1
Commercial Gums

Marine plants	Terrestrial plants	Microbial polysaccharides	Polysaccharide derivatives
Agar	Guar gum	Dextran	Carboxy methylcellulose
Alginates	Gum arabic	Gellan gum	Methyl hydroxypropyl cellulose
Carrageenan	Gum tragacanth	Rhamsan gum	Hydroxypropyl cellulose
Furcellaran	Karaya gum	Welan gum	Hydroxyethyl cellulose
	Locust bean gum	Xanthan gum	Propylene glycol alginate
	Pectin	Curdlan	Hydroxypropyl guar
	Tamarind seed gum	Pullulan	Modified starches

Gellan Gum as a Gelling Agent. Gellan gum is a polysaccharide polymer (**figure 1**) produced by the bacteria *Sphingomonas elodea* in nature. Companies, such as CPKelco, commercially produce gellan gum as and ingredient for the food, pharma, medical, and consumer products industries using industrial fermentation of pure *Sphingomonas elodea* cultures. Gellan is useful in many products because of its fast gelation properties and ability to form food gels. A food gel can be defined as "a high moisture three-dimensional polymeric network that resists flow under pressure and more or less retains their distinct structural shape (mechanical rigidity)" (Banerjee and Bhattacharya 2012). In food gels such as gellan gum gels, the structure is held together by weak molecular interactions between the polysaccharide polymers. Because of this mechanism, the gel's texture and rheological properties can be controlled by both the chemistry of the gellan polymer (e.g. removal of naturally occurring acyl groups) and the conditions (e.g. temperature, salt or sugar additives).

Gellan Gum Structure



The molecular structure of gellan gum is a straight chain based on repeating glucose, rhamnose, and glucuronic acid units[3,4]. In its native or high acyl form, two acyl substituents – acetate and glycerate – are present. Both substituents are located on the same glucose residue, and on average, there is one glycerate per repeat and one acetate per every two repeats[5]. In low acyl gellan gum, the acyl groups are removed completely. The acyl groups have a profound influence on gel characteristics. The high acyl form produces soft, elastic, non-brittle gels, whereas the low acyl form produces firm, non-elastic, brittle gels.

Figure 1. Structure of High Acyl and Low Acyl Gellan Gum.
Source: ("KelcoGel gellan gum book" 2007)

The presence and concentration of ions, particularly cations (such as Na^+ and Ca^{++}), can impact the gelation time, setting temperature and final rheological properties of the gums. The effect of divalent cations is larger than monovalent cations (**Figure 2**). In this lab, you will use a calcium solution to create beads of microencapsulated bacteria.

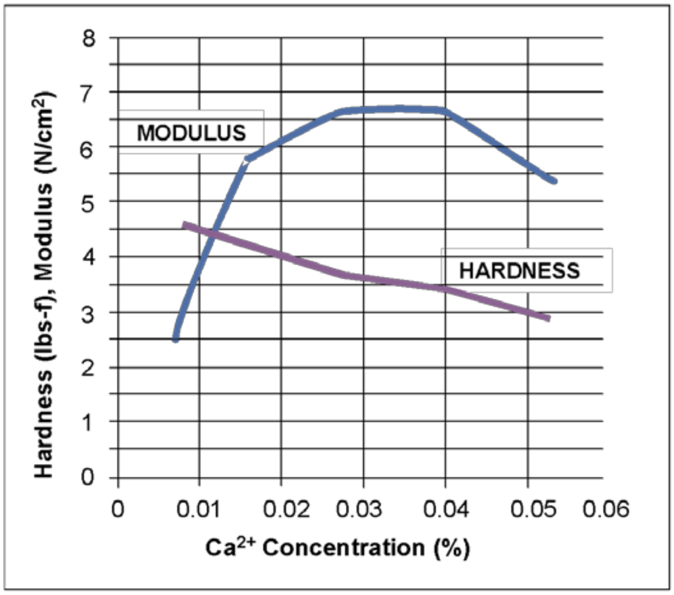


Figure 3. Calcium concentration alters the hardness and storage modulus of low acyl gellan gum (0.25%). Source: ("KelcoGel gellan gum book" 2007)

Equipment

- Heat plate with Stirring, plus stir-bars
- Waterbath (80 – 90C)
- Incubators set at 37C (for bacteria growth)
- Syringe
- Mass balance
- Beakers
- Filters or screens
- Invitrogen Evos Fluorescent Microscope
- Thermometers
- Small graduate cylinders (1 per group)

Supplies

- *Escherichia coli* GFP (ATCC® 25922GFP™)
    - http://www.atcc.org/Products/Cells_and_Microorganisms/Bacteria/Reporter_Labeled_Bacteria/25922GFP.aspx#documentation
- Tryptic Soy Broth (or LB broth), e.g. Sigma Aldrich, 800mL (43592-800ML, $79)
    - https://www.sigmaaldrich.com/analytical-chromatography/microbiology/microbiology-products.html?TablePage=18176790
- Ampicillin

- Xanthan Gum
- Low Acyl Gellan Gum
- High Acyl Gellan Gum
- 0.1 M calcium chloride ($CaCl_2$)
- DI water
- Disposable syringe pipetters

<u>PRE-LAB:</u>

Read the following paper (available in blackboard).

ELSEVIER

International Journal of Food Microbiology 61 (2000) 17–25

INTERNATIONAL JOURNAL OF
Food Microbiology

www.elsevier.nl/locate/ijfoodmicro

Survival of bifidobacteria in yogurt and simulated gastric juice following immobilization in gellan–xanthan beads

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Answer the following questions using complete, well written, thoughtful sentences.

1. What is the purpose of encapsulating the bacteria in this paper?
2. Why did the authors choose a combination of xanthan gum / gellan gum to encapsulate the bacteria?
3. What questions did the authors want to answer by conducting the study?

<u>EXPERIMENTAL DESIGN</u>

LAB Week 1. Gum Bead Formation and Characteristics

I. Conduct Preliminary Experiments. The goal of week 1 is for you to “get to know” the gellan / xanthan gum system.

Procedure.

1. Preheat water to 80C
2. You will make 4 different samples:
    a. 1% xanthan
    b. 0.75% gellan (high acyl)
    c. 0.75% gellan (high acyl) / 1% xanthan
    d. 0.75% gellan (low acyl) / 1% xanthan

3. Be sure to record the mass of gum and volume of water in each case.
4. Add gums to 80C water while stirring.
5. Stir for 1 hour.
6. Cast some of each sample into small beaker with a thermometer and let the gel cool.
    a. Does each sample form a gel as it cools?
    b. At what temperature does the sample gel?
    c. What is the “feel” of the gel? Is it goey, brittle, stiff?
    d. Does the gel flow when you apply force on it with your fingers or an instrument?
    e. When you remove the force, does the gel regain its original shape?
7. Create gel beads
    a. Using the needle syringe, drop gellan/xanthan beads into a 0.1M Calcium chloride solution
    b. Does your gel form beads when you drop it in?
    c. Using a screen or filter, pull out the beads.
    d. Observe your beads. Size, feel, color etc. Noting differences.
    e. Estimate the volume and diameter of your beads by volumetric displacement using a graduated cylinder.

LAB Week 2.

TBD Based on Experimental Design Developed by your group in Class.

References.

Banerjee, S., and S. Bhattacharya. 2012. 'Food gels: gelling process and new applications', *Crit Rev Food Sci Nutr*, 52: 334-46.

Burgain, J., C. Gaiani, M. Linder, and J. Scher. 2011. 'Encapsulation of probiotic living cells: From laboratory scale to industrial applications', *Journal of Food Engineering*, 104: 467-83.

Jaroch, D., E. McLamore, W. Zhang, J. Shi, J. Garland, M. K. Banks, D. M. Porterfield, and J. L. Rickus. 2011. 'Cell-mediated deposition of porous silica on bacterial biofilms', *Biotechnol Bioeng*, 108: 2249-60.

"KelcoGel gellan gum book." In. 2007. edited by CPKelco.