CHITIN CRAZE

Some scientists remain positively charged about chitin

By ELIZABETH PENNISI

Crab shells have it. Lobster and shrimp shells have it, too – as do butterflies, ladybugs, and all insects. Even mushrooms make the carbohydrate known as chitin.

Chitin is one of nature's most common organic compounds, second only to cellulose, a close cousin.

That cousin – a key structural carbohydrate in plant cell walls – has become one of the world's most utilized resources, primarily in the pulp and paper industry. Some entrepreneurs see similar potential in chitin.

For now, however, seafood processors treat the raw material as garbage. Each year, they cast off millions of tons of chitin-rich shells that pile up along coastlines around the world.

Chemically, both cellulose and chitin are polysaccharides – polymers, or long molecules, consisting of sugar molecules strung together. Oxygen atoms link each sugar's ring of carbon atoms to the next sugar's ring. Small groups of atoms, called side groups, hang off these rings.

A slight difference in one side group distinguishes chitin from cellulose, creating a whole new material for scientists to exploit.

In nature, chitin serves as a glue for the chemical components making up the delicate wings of insects and the crunchy coats of crustaceans. Over the past decade, researchers in Japan, Europe, and the United States have chemically modified this compound for use in bandages, burn dressings, food additives, drug capsules, and cosmetics (SN: 11/25/89, p.345). Scientists have even begun trying out a chitin compound as an edible film for preserving the quality and texture of foods (SN: 1/4/92, p.13).

Japan and Europe already use chitin – or, more often, a slightly modified and more versatile version called chitosan – in a variety of products. In the United States, however, economic and regulatory constraints have dampened initial interest in chitin's commercial potential.

But in recent years, the increased emphasis on safeguarding the environment has inspired a new generation of U.S. scientists to take a closer look at chitin and chitosan. The growing demand for nonpolluting products and for less wasteful industrial processes has made these two materials – both nontoxic, biodegradable substances – more appealing. Applications that appeared too costly five years ago today seem more attractive, especially in the field of waste treatment.

"Companies have become extremely worried about [waste] liability and disposal," explains Gregory F. Payne, a chemical engineer based at the University of Maryland in Baltimore.

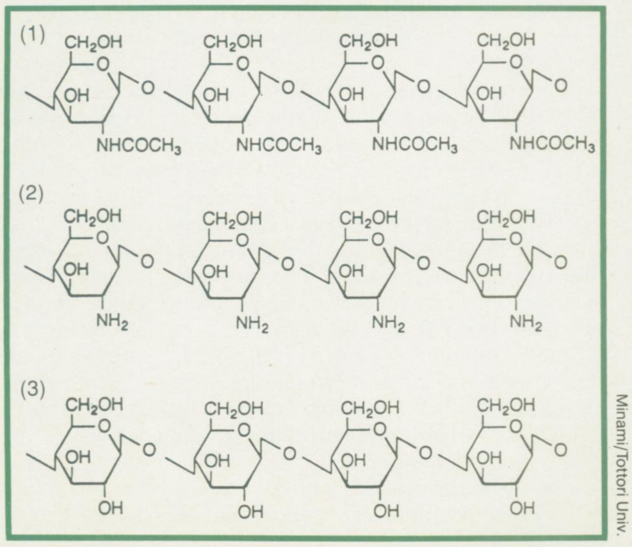
Because chitin normally cycles through the environment, decomposing naturally into its hydrogen, carbon, nitrogen, and oxygen building blocks, some researchers want to use it to help keep pollutants out of the environment. Others are investigating ways to improve chitin's potential as a biopolymer in medicines, paper, and filters. All of these researchers hope their work will make U.S. chemical companies and investors pay more attention to this plentiful resource and help promote its commercialization.

To obtain chitin from crab, lobster, or shrimp shells, researchers first dissolve the shells' calcium carbonate and then remove protein fragments, leaving behind chitin as a white powder. Dunking that powder in a concentrated sodium hydroxide solution heated to above 135°C removes one of chitin's side groups. This converts chitin to chitosan, a more manageable molecule that dissolves more readily.

In many ways, chitin seems a perfect raw material, almost too good to be true. For one thing, it qualifies as a renewable resource, generated as a waste product of shellfish processing or through the fermentation of fungi. Indeed, many states are concerned by the excessive landfill space taken up by bulky waste shells, notes Sam Hudson, a chemist at North Carolina State University in Raleigh.

Second, this abundant material seems to pose little threat as a toxic pollutant. Not only does it degrade naturally, but "it's a very biologically friendly material," says E. Ray Pariser, a biochemist at the Massachusetts Institute of Technology. He cites tests showing chitin to be nontoxic to the human immune system. In fact, chitosan fibers or granules applied to skin seem to keep fungi and microorganisms from growing there, he says. It even appears to stimulate the growth of beneficial bacteria in digestive tracts. Studies also suggest that when chitin-like chemicals break down in the body, their by-products can be used by the body. Such "biocompatibility" makes chitin desirable for medical applications, Pariser says.

Finally, chitin is not like most polysaccharides. "The properties are considerably different from those of other natural polymers," Payne says. Chitin's special identity comes from its amine side groups, ammonia-like molecules in



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Chemical cousins: (1) chitin, (2) chitosan, and (3) cellulose.

which an acetyl chemical group has replaced one of the two hydrogen atoms. By removing some acetyl groups, chemists create chitosan, a polymer with a strong positive charge – which most polysaccharides lack – and lots of options for researchers seeking to modify the molecule to impart new properties.

"You can do chemistry on the amine group or you can do chemistry on the hydroxyl [side group]," says William H. Daly, a chemist at Louisiana State University in Baton Rouge. "But it's the amine function that makes all the difference."

Because of its positive charge, chitosan readily forms films on negatively charged surfaces such as hair and skin. This makes it useful as an ingredient in cosmetics. Furthermore, several studies indicate that chitosan's charge helps it bind to fats and cholesterol, initiate clotting of red blood cells, and inhibit the formation of fibrin in wounds, reducing the formation of scar tissue.

That positive charge is what attracted research civil engineer Susan Murcott to chitosan. Working at MIT, she and Donald R.F. Harleman are seeking ways to improve the first stage of wastewater treatment in order to make later steps simpler and less costly.

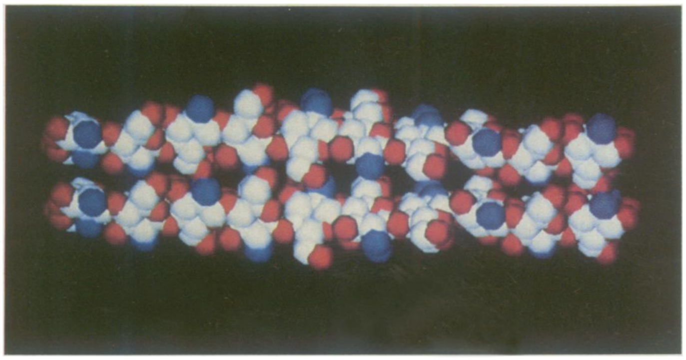
Typically, a little less than two-thirds of the suspended matter in sewer water settles out during this primary step. At the same time, the organic component, as measured in terms of oxygen utilization (biochemical oxygen demand), decreases by 35 percent. Then, during a second step, microbes break down most of the remaining organic matter.

The MIT engineers decided to try chitosan to increase the effectiveness of the initial step. In the laboratory and at a local sewage-treatment plant, Murcott tested whether chitosan could cause suspended matter to clump into bigger particles, which sink to the bottom of the treatment tank. Then, during the spring and summer of 1992, she and Harleman examined chitosan's ability to remove metals and polycyclic aromatic hydrocarbons (such as naphthalene used in mothballs) from wastewater.

In a relatively new process called chemically enhanced primary treatment, the chemicals alum and ferric chloride encourage clumping during initial processing and eliminate 15 to 20 percent more suspended matter than the standard primary treatment. Murcott and Harleman wanted to know whether chitosan could do even better than alum or ferric chloride. In general, it took about 13 percent as much chitosan as alum to remove 66 percent of the suspended matter, Murcott observed. Chitosan did not work as well when rainfall diluted the suspended matter, she notes. But at all times, the chitosan removed metals as well as other additives and yielded less sludge.

If chitosan could be obtained at a reasonable cost, says Murcott, then it could solve two environmental problems at once for places like Gloucester, Mass., a fishing town just north of Boston.

Each year, seafood processors in Gloucester generate tons of chitin-laden shells that need disposing. They also generate wastewater full of organic debris. Murcott hopes that chitosan from the shells will someday help clear the organic debris from the wastewater. But because chitosan currently comes from specialty chemical companies far from Gloucester, the high cost discourages its use for this purpose, she says. And so far, neither town officials nor entrepreneurs have thought it worth their while to create the infrastructure needed to collect chitin from Gloucester's processing plants and convert it to chitosan.



Attila E. Pavlath/USDA

Model of chitosan molecule.

Murcott recently began investigating chitosan's potential for removing particles from drinking water. As with the wastewater experiments, she first tested chitosan's potential in the laboratory, using water samples from a local reservoir. She discovered that chitosan alone did not work well. But in combination with a fine inorganic clay called bentonite, it helped clump suspended matter together so that it settled out more readily, quickly clearing up the water, she says.

Typically, municipal water departments use aluminum salts to clarify water and make it colorless, generating a sludge containing a residue of metal salts. Tougher environmental regulations make the disposal of that sludge a growing problem, says Murcott. Synthetic polymers used in place of aluminum salts also raise concerns because of their possible toxicity. Consequently, the low amounts of chitosan needed, the low volume of sludge it generates, and chitosan's biodegradability make it a desirable alternative, she says.

Last year, Payne and graduate student Wei-Qiang Sun began to harness the reactivity of chitosan's amine group to help streamline chemical manufacturing. Many industrial processes – including the making of flavors, fragrances, pharmaceuticals, and plastics – involve or generate contaminating chemicals called phenols somewhere along their production pathways. Once those phenols mix with unreacted starting chemicals, the starting chemicals cannot easily be cycled back to the beginning of the processing pathway. For this and other reasons, the phenols reduce the efficiency of these processes.

At the spring meeting of the American Chemical Society, held in Denver last March, Payne and Sun described their technique for removing phenols. It entails two steps, initiated by adding two ingredients to the watery bath in which a fragrance or other chemical is produced. The Maryland researchers use an enzyme called tyrosinase, derived from mushrooms, to link oxygen with any phenol molecules. This reaction converts each phenol to a compound called a quinone. Then they add chitosan to filter out the quinones.

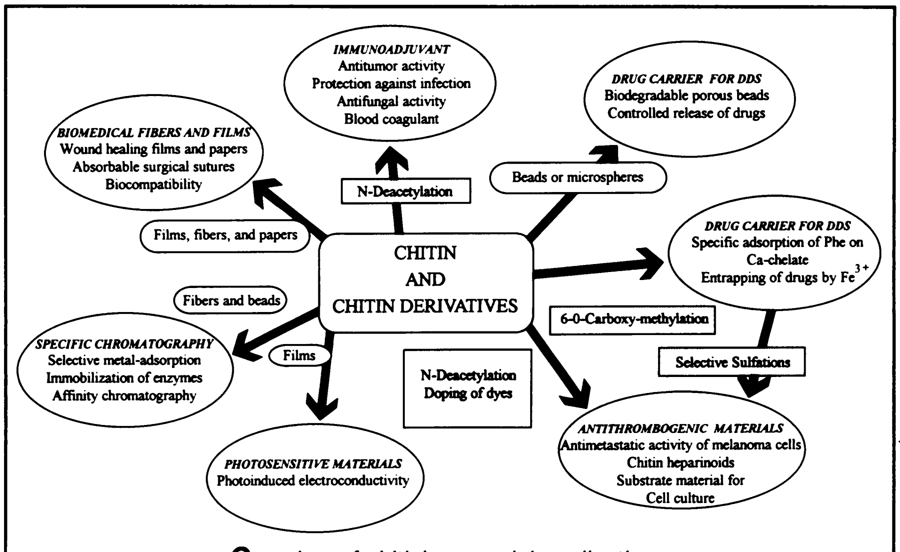
Payne sees two advantages in this process. For one, the quinones link more readily with chitosan than phenols do. In fact, chitosan works better than activated charcoal, another absorbent filter, he says. Second, "tyrosinase is very specific and won't destroy any of the intermediate compounds," he says.

Already, Payne and Sun have tested their approach in the processing of a half-dozen hydrogen-carbon compounds, and tyrosinase reacted with only the phenols. "We're specifically pulling out the contaminating phenols," Payne says.

Companies that generate many tons of waste phenols remove this contaminant with a distillation step. But the equipment needed for that approach costs too much to be practical for manufacturers of specialty chemicals, including pharmaceuticals, he notes. That's where this two-step method can come in handy and may prove cost effective.

"We think [chitosan] will have unique applications for industrial waste minimization," Payne says.

Hudson has also demonstrated chitin's potential for making manufacturing processes more environmentally friendly. In laboratory tests at North Carolina State, he and Brent Smith discovered that chitin can absorb most dye from water. This suggests that textile companies can use it to decolor water generated by the production of colored fabrics, Hudson says. Chitin can absorb up to 20 percent of its weight in dye molecules, he adds.



Overview of chitin's potential applications.

Minami/Tottori Univ.

Chitin's proponents emphasize that it can do much more than treat wastewater. The list of potential uses includes applications in agriculture, in separation and purification technology, and as a fiber for paper, packaging, even clothing, says Hudson. And scientists keep churning out more possibilities:

• In Japan, veterinarians have confirmed that chitosan aids healing and helps fight infection. Because veterinarians tend to confine wounded animals during their recovery, the animals face a greater risk of infection from dirt or feces that can accumulate in these tight quarters. Last year, Saburo Minami of Tottori University reported that he had applied cotton made of chitosan fibers to complicated wounds of 200 cows, horses, dogs, and cats. More than 80 percent of the animals recovered, sometimes with quite dramatic results in comparison with animals not treated with chitosan, Minami says.

In addition, Minami found that treatment with chitosan "cotton" reduced the number of times wounded animals needed to receive antibiotics during their recovery. He also used local injections of chitosan granules to speed the recovery of cows suffering from udder infections.

Minami's studies of recovering tissue indicate that chitosan causes white blood cells to gather rapidly at the site, where they help subdue infection, he says. "The phenomenon is very useful and hopeful for humans," asserts Minami, who thinks chitosan dressings will prove particularly helpful in combating bedsores.

• Chitosan can make bones heal faster, says Gregory Borah, a plastic surgeon at the Robert Wood Johnson Medical School in New Brunswick, N.J. In 1991, he reported success in using chitosan compounds to help heal holes in the leg bones of rodents. He has since expanded his testing to jaw and skull bones. In his experiments, Borah makes a hole too large to heal on its own in a bone on each side of the rat. He then adds the chitosan compound to one side. New bone forms 95 percent of the time on that side and never on the untreated side, he told SCIENCE NEWS. "We're really shocked about [this] positive response," he added.

As a long, charged polymer, chitosan attracts white blood cells called macrophages to the bone, Borah says. These cells may liberate messenger chemicals that cause immature bone cells nearby to mature into specialized bone cells, he suggests.

• In the 1980s, freelance agriculturalist Don Freepons of Kennewick, Wash., discovered that coating rice seeds with chitosan leads to higher yields of rice. For the past four years, he has worked with Richard Dunand of Louisiana State University's Rice Research Station in Crowley, testing different grades of chitosan for enhancing rice production.

Their experimental plots yield 5 to 8 percent more rice, Dunand told SCIENCE NEWS. In one test, rice seeds coated with the equivalent of 40 grams of chitosan per acre yielded 308 pounds more rice than did uncoated seeds grown under the same conditions, he says. The experimental fields average 4,350 pounds of rice per acre.

"With the evidence we're generating here, [chitosan] will have a benefit to the rice farmer," Dunand asserts. He adds, however, that he and Freepons aren't sure how chitosan causes this effect, because the variability among individual plants makes it difficult to document subtle differences in the number of stalks or in the number of rice grains per stalk.

• Harvard University researchers are developing a chitosan compound that may help halt the spread of AIDS. Ruth M. Ruprecht and her colleagues modified chitosan by adding sulfate to the polymer's repeating units. That compound slowed the synthesis of proteins by the AIDS virus in cultured mouse and human cells—yet the cells remained healthy, the researchers reported in the Jan. 31, 1991 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS. Further experiments demonstrated that the chitosan compound can keep the virus from attaching to cell surfaces and can interfere with the activity of a key viral enzyme called reverse transcriptase. The Harvard team has continued that work but has not yet published new results, Ruprecht told SCIENCE NEWS.

With such potential, why isn't chitin a household word? "The chitin business has sort of been a mom-and-pop operation," Daly explains. "[Chemical] companies have not made a major commitment to produce a reliable supply."

"You can't scale up anything that involves chitin unless you have an absolutely solid source of chitin," adds Karen Dean, editor of INDUSTRIAL BIOPROCESSING, a monthly newsletter published by Technical Insights, Inc., in Fort Lee, N.J. The United States has no single seafood-processing center, and shellfish harvests vary with the year and season. "Just ensuring a source of material and a market for what you make with it is really tough," Dean says.

And even a sure, continuing source might be insufficient. Chitosan need not be perfectly pure for treating wastewater, but other applications demand that every molecule be exactly alike, since even trace amounts of contaminants can cause concern. Yet only since 1991 have companies begun discussing the development of specific standards for grades of the material, Borah says.

"If you want to use chitin for biomedical purposes, you have to get rid of all the junk. Even fractions of a percent are enough to make the FDA nervous," adds Pariser. In a crustacean shell, chitin binds tightly to trace elements and proteins encountered in the environment. Consequently, the composition of chitin can vary depending on where the animal lived. Pariser thinks he has developed a way to get around this problem, but he doesn't want to talk much about it yet.

"I think once we get patent protection and all the results are in, this [process] will drastically change the whole [situation] with chitin," he says.

Meanwhile, other chitin aficionados will continue their efforts to turn chitin into a versatile commodity, but with expectations much more realistic than those of their predecessors, who boasted that by the 21st century, this material would be the basis for a billion-dollar industry.

"It's an interesting material," says Borah. "But everybody seems to have a hard time getting it into the end zone." □