material. It may be assumed, therefore, that in nature where conditions are not static, but constantly fluctuate within the optimum conditions for growth of this bacterium, the amount of acid attributable to such bacterial activity may be of tremendous importance.

SUMMARY

Some as yet unclassified bacteria isolated from bituminous coal mine effluents were tested for oxidation of ferrous iron as well as sulfur contained in "sulfur ball" material and museum grade marcasite and museum grade pyrite. These bacteria oxidize ferrous iron in acid solutions. The bacteria produced acid and formed sulfate from sulfur ball material and museum grade marcasite but had no effect on pyrite. Acid production by bacteria may, in part, cause the high acidities encountered in many bituminous coal mine effluents.

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A Continuous Feeding Assembly for Laboratory Fermentations

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Laboratory shakers generally available for fermentation studies are suitable for experiments wherein all nutrients are supplied initially or in several large fractions. Since continuous or increment feed schedules are essential in the production of bakers' yeast, an appropriate assembly was designed and constructed for operation with a Gump rotary shaker. Its satisfactory, low-cost operation during the past two years has prompted a description of component parts to promote its adaptation to other applications.

The increment-fed shaker system, shown in figure 1, is comprised of two principal parts: the shaker, and the superstructure bearing the feeding unit with actuating cams driven by a synchronous motor through a reducing gear assembly.

On the platform of a Gump shaker are fixed 40 metal, rubber-lined seats to fit one-liter Florence flasks which are held firm by wooden braces anchored to the platform with a bolt and wing nut arrangement. The pyrex culture flasks are indented at selected points to achieve effective aeration. With the shaker operating at 240 rpm, our medium with a flask having eight indentations shows an oxygen uptake of 0.2 mM O_2/liter/min as determined by sulfite oxidation. A flask with 30 indentations under the same conditions results in an uptake of 5.3 mM O_2/liter/min.

The solution to be fed into the culture flask is held in a standard 50 ml syringe containing a small stainless steel spring to oppose the fall of the barrel. Pressure from the feed bar motivated by the cam gang forces the solution through the needle of the syringe into a thistle tube fitted with flexible tubing for delivery to the flask on the shaker platform.

The cams are driven by a "Master" Gearmotor[1] with a coordinated shaft. This motor is located on a movable platform to permit easier disengagement and realignment of the cam arrangement at the start and at selected intervals of an experiment. Cam design is dependent upon the rate of feed desired and the capacity of the syringe serving as a reservoir for the nutrient solution. The cams shown in figure 1 permit

[1] The Master Electric Co., Dayton, Ohio.

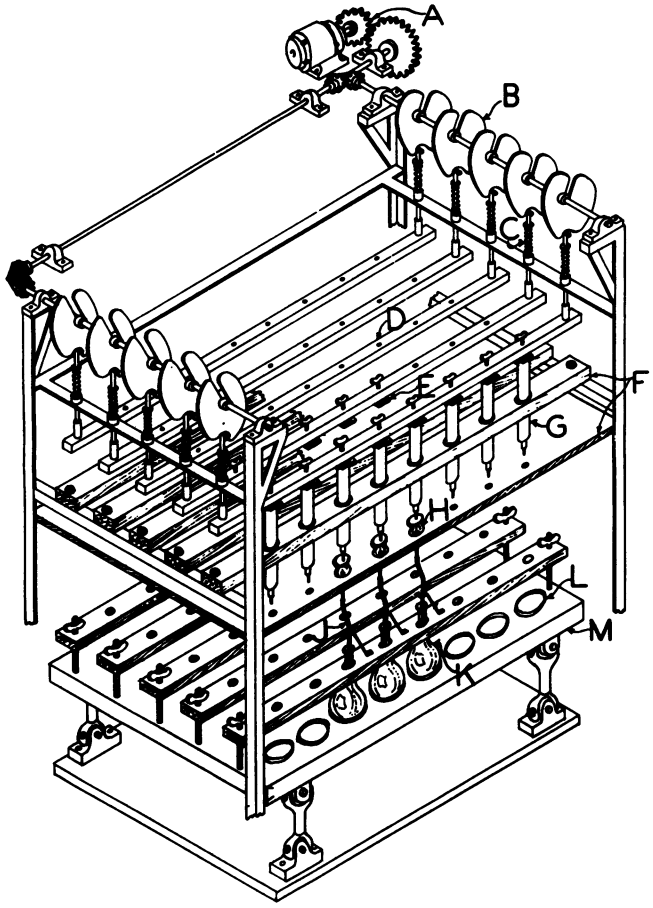

FIG. 1. Continuous feeding system: (A) Gearmotor, (B) Cam gang, (C) Cam follower, (D) Feed bar, (E) Adjustable contact point, (F) Syringe rack, (G) Syringes, (H) Thistle tube, (J) Flexible tubing, (K) Culture flasks with indentations, (L) Lined seat, (M) Shaker table.

a 20-hour interrupted cycle with total delivery of 50 ml of nutrient solution.

Operating procedure for a typical bakers' yeast fermentation is given for illustrative purposes. Flasks are charged with the seeded medium and fastened to the shaker platform. Thistle tubes are inserted into holes in the platform and connected with flexible tubing, the free ends placed temporarily into one of several waste receptacles until final adjustment of syringe tension is made. Syringes assembled with springs are filled with nutrient solution, needle attached, air excluded, and mounted in the syringe rack; the movable unit consisting of the thistle tube receiver and syringe rack is slid under the feed bar. The cam assembly is turned manually until the cam followers are at the zero position of the cam; and then the motor gears are engaged with the cam shaft gear. Thumb screws on the feed bar are adjusted to assure contact with the top of the syringe barrel. After flushing the thistle tube and delivery lines with sterile water, the delivery tubes are inserted into the flasks and secured by means of alligator clips. Shaker and cam motors are started simultaneously.

Although designed for operation as a quasi-sterile open fermentation system, minor alterations of some components of the feeding arrangement will permit satisfactory continuous or increment-fed fermentations in a closed or aseptic system.

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SUMMARY

A cam driven continuous feeding assembly is described which has been employed successfully in laboratory study of yeast growth in agitated flasks.