Vinasse

Crops

Identification of Petitioned Substance

Chemical Names:
Vinasse (cane-, beet-)

Other Names:
Molasses vinasse
Stillage
Molasses stillage
Distillery wastewater
Distillery slops
Cane molasses solubles (CMS)

Trade Names:
ProLico®
Bio-Vinasse

CAS Numbers:
91082-90-5
No specific CAS for cane/beet vinasse

Other Codes:
European Inventory of Existing Commercial Chemical Substances (EINECS) No. 293-805-3

Summary of Petitioned Use

The petition before the National Organic Standards Board (NOSB) is to add vinasse (beet-, cane-) as an allowed synthetic substance in organic crop production (§ 205.601) for the purpose of augmenting soil nutrient levels. There are no regulations promulgated by the National Organic Program (NOP) regarding the use of vinasse in organic livestock production or handling from which comparisons may be drawn. Although vinasse (beet-, cane-) has a long-standing history of various agricultural applications outside of the U.S., the use of vinasse as a fertilizer for organic crop production necessitates consideration of the chemistry of its component chemicals and residues in terrestrial and aquatic environments.

Characterization of Petitioned Substance

<u>Composition of the Substance:</u>

Vinasse is the principal byproduct generated during the distillation of ethyl alcohol, yeast, amino acids, and/or organic acids from cane and beet molasses fermentation mixtures. As such, it contains every substance added to the fermenter, plus yeast metabolites and yeast cell contents (Willington, 1982). The primary organic and inorganic chemical components of vinasse are proteins, organic acids, amino acids, unfermented carbohydrates, vitamins, and minerals (Hidalgo, 2009). In particular, high concentrations of potassium, calcium, magnesium, sulfur, and nitrogen are typically found as components of vinasse, which makes it particularly attractive as a soil amendment/fertilizer. Glycerol, lactic acid, ethanol, and acetic acid (all byproducts of the fermentation process) are the major organic compounds found in cane and beet vinasse. The principal anions present are sulfate and chloride, with molasses stillage (i.e., distillation residue) having a higher salt loading than other stillages (Willington, 1982). Information regarding the characterization and elemental composition of cane and beet vinasse is summarized below in Table 1 (España-Gamboa, 2011).

<u>Source or Origin of the Substance:</u>

Vinasse is generally obtained through distillation of fermented cane and beet molasses, which is a byproduct of cane and beet juice processing for the production of pure or refined sugar. In brief, the process of vinasse production commences with the harvesting of sugar cane and sugar beets. The sugarcane or sugar beet is subsequently processed, separating cellulosic components from the cane or beet juice. Sulfur dioxide is sometimes added during the processing of beet juice prior to crystallization to decolorize the cane juice. Multiple crystallization procedures are conducted on the syrup made from the juice after clarification to produce sugar crystals, which are separated from the molasses by centrifugation and then harvested. The resulting byproduct, molasses, is then mixed with yeast or other microorganisms and fermented. Depending on the yeast or microorganism used, fermentation converts the carbohydrates (i.e., sugars) contained within the molasses to ethyl alcohol, organic acids, and/or other desired organic compounds. In the case of ethanol production, small amounts of sulfuric acid may be added prior to fermentation to reduce the populations and activity of undesired bacterial species by adjusting the pH to between 4 and 5. Distillation of the resulting fermentation broth separates the desired organic compounds (e.g., ethanol) from the mother liquor. Vinasse is the byproduct of the distillation procedure, with 9–20 liters of vinasse generated per liter of ethanol (Parnaudeau, 2008; España-Gamboa, 2011). Please see Evaluation Question #2 for more information regarding the production methods of vinasse, including the use of synthetic and non-synthetic materials in the industrial process.

Properties of the Substance:

The petitioned substance (i.e., vinasse, stillage) is a liquid of low viscosity at 25 °C, having a very dark brown color and weakly caramel, non-pungent odor (Tate & Lyle, 2005; DCM, 2010). It is also slightly acidic, having a pH of approximately 4.5–4.8, which may vary depending on the natural source and/or production method employed. Vinasse is an aqueous solution containing both organic (e.g., carbohydrates, proteins, and vitamins) and inorganic (e.g., nitrogen, sulfur, and minerals) compounds; as such, it is denser than water at room temperature (vinasse density = 1.25–1.33 g mL^{-1}; water density = 1.0 g mL^{-1}). Vinasse is infinitely soluble in water (Tate & Lyle, 2005; DCM, 2010). Although there is no vapor pressure reported for vinasse, volatilization of low molecular weight organic components such as lactic acid and acetic acid from soils treated with vinasse is likely.

Table 1. Composition and yield of vinasse from cane and beet molasses

	Vinasse from Cane Molasses	Vinasse from Beet Molasses
BOD (g L^{-1})	39.5	27.5–44.9
COD (g L^{-1})	84.9–95	55.5–91.1
Nitrogen total (mg L^{-1})	153–1230	1800–4750
Phosphorus total (mg L^{-1})	1–190	160–163
Potassium (mg L^{-1})	4893–11000	10000–10030
Sulfate total (mg L^{-1})	1500–3480	3500–3720
pH	4.46–4.80	4.30–5.35
Copper	0.27–1.71 mg L^{-1}	2.1–5.0 mg kg^{-1}
Cadmium	0.04–1.36 mg L^{-1}	<1 mg kg^{-1}
Lead	0.02–0.48 mg L^{-1}	<5 mg kg^{-1}
Iron	12.8–157.5 mg L^{-1}	203–226 mg kg^{-1}
Phenols	34 mg L^{-1}	450 mg kg^{-1}
Vinasse Yield (L $L_{ethanol}^{-1}$)	12–20	9–15

BOD = Biochemical Oxygen Demand; COD = Chemical Oxygen Demand; Source: España-Gamboa, 2011

Specific Uses of the Substance:

The petitioned use of the substance is as a plant nutrient/soil amendment in organic crop production (BioBizz Worldwide, 2011). All of the available literature included in this report for agricultural uses of vinasse originated from outside of the U.S. Vinasse obtained from cane and sugar beets is commonly used as a fertilizer in certain countries for conventional agriculture due to its nutrient content, particularly calcium, potassium, and organic materials, and the vast quantity of vinasse generated during ethanol production (España-Gamboa, 2011). Application to crops typically involves dilution of a commercially available vinasse concentrate with water, and subsequent spraying of crops with the diluted aqueous solutions. Depending on soil and plant nutrient requirements, vinasse may be applied as a 1–2% diluted solution to land (80–160 liters per acre) through irrigation or foliar spray for direct absorption by the plant. Vinasse may also be used as a compost ingredient. When applied in these ways, vinasse is expected to:

- Enrich soils with macro-nutrients (e.g., nitrogen, phosphorus, sulfur),

- Enrich soils with micro-nutrients (e.g., vitamins and trace minerals),
- Maintain soil organic carbon levels,
- Enhance micro flora and fauna development within soils,
- Improve the nutritional profile of crops for human and animal consumption,
- Enhance the growth potential of crops.

The product information sheet for ProLico®, a vinasse-containing product manufactured in Belgium and used in Europe, states the following applications: "in soils or substrates for horticulture, tree nurseries, amenity planting, and landscaping." Suggested dosages are 1–2 g L^{-1} and 3–5 g L^{-1} of nutrient solution for continuous and periodic application, respectively (DCM, 2010). Exact dosages depend upon the crop, time of application (cultivation phase), the soil's nutritional reserve and intensity of sprinkling. Research and development of fertilizers made from vinasse is an active field; for example, the patent literature describes a method for the recovery of industrial water, decontamination and drying of vinasse using a micronizing procedure and formulation of the resulting material into an organic mineral fertilizer (Invitti, 2012).

Vinasse is also used as an additive or feed supplement for conventional ruminant and non-ruminant livestock (Hidalgo, 2009). Recent studies have indicated significant decreases in the costs of animal feed and more efficient production outcomes when using beet vinasse in livestock production (Hidalgo, 2011). In addition, a positive correlation was observed between consumption of vinasse and animal behavior, most likely due to the high content of organic acids and B complex vitamins contained within vinasse. Researchers incorporating small amounts of vinasse into the diets of chickens being fattened for meat production reported enhanced live weight conversions and increased weights for carcass and edible parts (Hidalgo, 2011). Further, these studies indicate that the inclusion of vinasse in hen diets led to greater viability.

Alternative applications of distillery vinasse are numerous and varied. Potential applications of vinasse include the biotechnological production of single cell proteins and organic acids (Wilkie, 2000). Calcium magnesium acetate is an organic acid salt generated through the fermentation of carbohydrates in vinasse, and may be used as an alternative di-icing agent for roadways (Wilkie, 2000). Recently developed production methods for enzyme synthesis utilize the nutrient components of vinasse; for example, the combination of vinasse and bagasse (the fibrous matter that remains following sugarcane processing) is a useful substrate for the microbiological production of cellulolytic and lignolytic enzymes (Aguiar, 2010). A modified laboratory-scale upflow anaerobic sludge blanket (UASB) reactor was developed for the production of methane from vinasse (España-Gamboa, 2012). Post treatment, the organic loading is significantly diminished, making the processed vinasse safe for agricultural use with diminished risk of polluting soil, underground water, or nearby aquatic systems. It should be emphasized that this method utilizes genetically modified microorganisms (GMMs) bearing recombinant DNA.

Vinasse disposal options must be utilized in the absence viable applications for the accumulated stillage. The aerobic degradation of beet molasses fermentation wastewater (vinasse) has been carried out using fungi of the genus *Penicillium* and *Aspergillus* (Jiménez, 2003). Significant decolorization of the waterwaters was observed following microbial treatment, and chemical oxygen demand (COD) reductions were observed for all four microorganisms employed. Vinasse has also been processed through combustion and incineration to an ash, which may be used as fertilizer (Cortez, 1997; Willington, 1982). Early means of vinasse treatment and disposal involved concentration of the stillage, neutralization with alkali, followed by incorporation into road building material (Wilkie, 2000). Distilleries in countries lacking wastewater effluent regulations or enforcement mechanisms frequently dispose of stillage in adjacent bodies of water.

<u>Approved Legal Uses of the Substance:</u>

Although some countries allow vinasse to be sprayed directly onto sugarcane crops as a fertilizer, the Renewable Fuel Standard Program Regulatory Impact Analysis document states that this practice is not currently allowed in the United States (U.S. EPA, 2010). However, restrictions on the use of vinasse as a fertilizer were not found in the Code of Federal Regulations. Environmental legislation prohibits inappropriate disposal of vinasse into rivers, lakes, the ocean, and soils due to the high nutrient loading and potential for polluting waterways (U.S. EPA, 2010). While vinasse is not specifically mentioned, 40 CFR 409 sets standard effluent limitations for beet sugar processing, crystalline sugar refining, and liquid cane sugar refining (40 CFR 409.10–409.37), in addition to other effluent regulations. For example, from 40 CFR 409 Subpart A (Beet Sugar Processing):

> *The limitations establish the quality or quantity of pollutants or pollutant properties, controlled by this section, which may be discharged by a point source subject to the provisions of this subpart after application of the best practicable control technology currently available; provided however, that a discharge by a point source may be made in accordance with the limitations set forth in either paragraph (a) of this section exclusively, or paragraph (b) of this section exclusively*

Certain characteristics and limitations of the effluent, such as Biochemical Oxygen Demand (BOD = 3.3 kg per 1000 kg of product, or 3.3 g L^{-1} of product), pH (within the range of pH = 6–9), fecal coliform levels, and temperature, are defined in subparagraphs (a) and (b). Specifically, these subparagraphs describe acceptable wastewater discharge resulting from barometric condensing operations and any other beet sugar processing operation.

The U.S. Food and Drug Administration (U.S. FDA) makes no mention of cane or beet vinasse for use as a livestock feed supplement; however, organic and inorganic chemical components of vinasse, such as acetic acid, lactic acid, and calcium and potassium salts, are listed as "Substances Generally Recognized as Safe" (21 CFR 582). Caramel is prepared through the carefully controlled heat treatment of molasses, and is exempt from certification as a color additive under 21 CFR 73. Further, 21 CFR 501 states that distiller solubles may be used in animal feeds listed as processed grain:

> *(b) Each collective name referred to in this paragraph may be used for the purpose of labeling where one or more of the ingredients listed for that collective name are present. The animal feed ingredients listed under each of the collective names are the products defined by the Association of American Feed Control Officials. The collective names are as follows:*
>
> *(5) <u>Processed grain byproducts</u> include one or more of the following: Brans, brewers dried grains, distillers grains, **distillers solubles**, flours, germ meals, gluten feeds, gluten meals, grits, groats, hominy feeds, malt sprouts, middlings, pearled, pollishings, shorts, and what mill run.*

Collective names may be used if the animal feed is intended solely for the production of livestock and poultry. Although cane and beet vinasse are not specifically referenced here, the composition of distillers solubles is likely to be comparable.

<u>Action of the Substance:</u>

Vinasse functions by enhancing the nutrient quality of soils and therefore crop production capacity. Some reported advantages of using vinasse include increased pH and cation exchange capacity, improved soil structure, increased water retention, and development of micro flora and fauna within the soil environment. The results of studies conducted over 15 years indicate that there are no damaging impacts on the soil at lower application doses (300 m^3 ha^{-1}), while higher doses may impair sugarcane viability. When applied to sandy or shallow soil, vinasse usage may lead to groundwater contamination (Zuurbier, 2008).

A number of scientific studies have analyzed the fertilizing efficiency of vinasse. For example, it was found that sugar and cane yields increased with application of vinasse, with the optimal results obtained through application of vinasse at the moderate dosage level of 50,000 L ha^{-1}. Further, the N (nitrogen), P_2O_5 (phosphorus) and K_2O (potassium) sugarcane demands were supplied at this dosage level in proportions of 55%, 72%, and 100%, respectively (Gómez, 2000). It was also observed that application of vinasse to sandy loam soil enhanced the organic carbon, available nutrient levels, and the microbial soil population, thereby resulting in higher sugarcane yields (Baskar, 2005). This report also suggested that application of distillery effluent 40–60 days prior to planting may overcome any issues with the excessive BOD of vinasse. The application of fresh beet vinasse had a detrimental impact on the soil's physical, chemical, biological properties and wheat yield parameters, most likely because high quantities of sodium and fulvic acids were introduced into the soil by vinasse. However, co-composting beet vinasse with cotton gin crushed compost beneficially impacted soil properties and increased wheat yield (Tejada, 2006).

Improper application of vinasse to agricultural land may lead to environmental impairment due to its potentially high organic loading, low pH and high salinity levels. As a result of the high levels of organic content, spreading of vinasse on soils may lead not only to anoxia but also alterations of mineral forms (i.e., complexation and solubilization) and metal mobilization (Lahlah, 2009). Although vinasse is typically applied to soils, the residue is able to enter water bodies via runoff and leaching. Its dark coloring, organic matter content, and low pH may potentially exert toxic effects on aquatic environments. As with other highly colored effluents, vinasse may exert negative impacts on the environment due to the inhibition of aquatic plant growth (Botelho, 2012). The effects of vinasse runoff on fish species were recently investigated, revealing high median lethal concentrations (LC_{50}) of vinasse (low toxicity) following adjustment of the vinasse pH from acidic to neutral: 2.99% (*Ceriodaphnia dubia*), 5.62% (*Daphnia magna*), and 8.34% (*Danio rerio*). Significantly higher mortality rates (and correspondingly lower LC_{50} values) were observed for vinasse prior to pH adjustment, suggesting that certain environments (e.g., lakes and ponds) may have a higher risk for aquatic toxicity from vinasse residue runoff (Botelho, 2012).

Combinations of the Substance:

Commercial vinasse concentrates are generally diluted with water and sprayed directly onto crops and soil. Synthetic fertilizer ingredients (e.g., nitrogen, P_2O_5, and K_2O) may also be applied in combination with vinasse, depending on soil nutrient requirements and the nutrient profile of the vinasse being used (Gómez, 2000). Otherwise, no additional ingredients (e.g., inert ingredients, stabilizers, preservatives, carriers, anti-caking agents, or other materials) are added to commercially available forms of vinasse.

Status

Historic Use:

Historically, vinasse has found agricultural applications as an additive for feed supplement for the feeding of ruminant and non-ruminant animals in several tropical countries and Europe. In addition, vinasse has been used as a fertilizer since the beginning of the 20th century (Hidalgo, 2009). Please see the "International" section below for details regarding the allowed use of vinasse in the organic production of crops and livestock in other countries.

Organic Foods Production Act, USDA Final Rule:

The Petitioned Substance, vinasse for use in crop production, is not listed within the Organic Foods Production Act of 1990 (OFPA) or the National Organic Program Final Rule, 7 CFR Part 205. However, as found in 7 CFR 205.205, organic crop producers must implement a crop rotation including but not limited to sod, cover crops, green manure crops and catch crops that provide for soil organic matter maintenance and improvement as well as management of deficient or excess plant nutrients. More specifically, 7 CFR 205.203 states that the organic producer must take the following actions: (1) Select and implement tillage and cultivation practices that maintain or improve the physical, chemical, and biological condition of soil and minimize soil erosion; (2) manage crop nutrients and soil fertility through rotations, cover crops, and the application of animal materials; (3) manage plant and animal materials to maintain or improve soil organic matter content in a manner that does not contribute to contamination of crops, soil, or water by plant nutrients, pathogenic organisms, heavy metals, or residues of prohibited substances.

International

European Economic Community Council

European Union regulations allow the use of vinasse as a fertilizer in organic crop production. Article 3(1) of Commission Regulation (EC) No 889/2008, allows the use of substances listed in Annex 1 as a fertilizer and soil conditioner in organic crop production when the nutritional needs of plants cannot be met by tillage and cultivation practices, crop rotation, and the application of livestock manure and/or organic material, as defined in Article 12(1)(a), (b), and (c) of Regulation (EC) No 834/2007. Specifically, Annex 1, regarding fertilizers and soil conditioners as referred to in Article 3(1), specifies the use of vinasse and the following related substances as fertilizers:

- Stillage and stillage extracts for use in organic crop production, excluding ammonium stillage

- Composted or fermented mixtures of vegetable matter – products obtained from mixtures of vegetable matter, which have been submitted to composting or to anaerobic fermentation for biogas production
- Industrial lime, obtained from sugar production or as a byproduct of sugar production from sugar beet.

European regulations also specify the use of products related to vinasse in organic livestock production. Article 22 states that "non-organic feed materials of plant and animal origin may be used in organic production …if they are listed in Annex V." From Annex V, Feed materials referred to in Article 22(1), (2) and (3), the following substances related to vinasse may be used:

- Tuber, roots, their products and by-products: Sugar Beet Pulp (Section 1.4)
- Other plants, their products, and by-products: Molasses (Section 1.7).

Article 22 (4) states that "feed additives, certain products used in animal nutrition and processing aids may be used in organic production only if they are listed in Annex VI." Under section 3 of Annex VI, sugar, sugar beet pulp, and molasses are listed as substances allowed for silage production.

Canadian General Standards Board

In comparison to European regulations, Canadian standards allow the application of vinasse as an organic fertilizer or soil amendment without specific restrictions on use. Section 4.2 (soil amendments and crop nutrition) of the Canadian organic production systems and permitted substances lists (CAN, 2011b) catalogues the following substances as allowed in organic crop production:

- Stillage and stillage extract, except ammonium stillage is prohibited
- Molasses, shall be organic molasses unless not commercially available
- Sugar, organic sugar may be used as an ingredient in a crop production aid

Codex Alimentarius

Vinasse is also allowed under the Codex Alimentarius Commisson guidelines of organically produced foods. *Annex 2: Permitted substances for the production of organic foods*, lists the following pertinent substances in Table 1, substances for use in soil fertilization and conditioning:

- Stillage and stillage extract, except ammonium stillage is excluded
- Byproducts of the sugar industry (e.g., vinasse); the need must be recognized by the certification body or authority

In addition, Table 4 in Annex 2 lists processing aids that may be used for the preparation of products of agricultural origin referred to in section 3 of the Codex guidelines. Included in this list are sulfuric acid for pH adjustment of extraction water in sugar production and sodium hydroxide for pH adjustment in sugar production.

International Federation of Organic Agriculture Movements

The IFOAM norms do not specifically allow the application of vinasse as an organic fertilizer or soil amendment. Products derived from sugar and sugar beet processing, including lime from sugar processing and sugar beet lime, are permitted in appendix two of the IFOAM norms as calcium and magnesium soil amendments. In addition, sulfuric acid is allowed as a processing aid for pH adjustment of water during sugar processing, and sodium hydroxide is also listed as allowed for sugar processing.

Evaluation Questions for Substances to be used in Organic Crop or Livestock Production

<u>Evaluation Question #1: Indicate which category in OFPA that the substance falls under:</u> (A) Does the substance contain an active ingredient in any of the following categories: copper and sulfur compounds, toxins derived from bacteria; pheromones, soaps, horticultural oils, fish emulsions, treated seed, vitamins and minerals; livestock parasiticides and medicines and production aids including netting, tree wraps and seals, insect traps, sticky barriers, row covers, and equipment cleansers? (B) Is the substance a synthetic inert ingredient that is not classified by the EPA as inerts of toxicological

concern (i.e., EPA List 4 inerts) (7 U.S.C. § 6517(c)(1)(B)(ii))? Is the synthetic substance an inert ingredient which is not on EPA List 4, but is exempt from a requirement of a tolerance, per 40 CFR part 180?

(A) Vinasse contains sulfur in the form of sulfate and sulfur-based organic compounds (e.g., amino acids). In addition, small amounts of copper compounds, vitamins, and minerals are typically observed in cane, beet, and other forms of vinasse. Copper and lead, in addition to other heavy metals, may be introduced via uptake from soils by the sugarcane or sugar beet feedstock. The corrosion of piping, tanks, and heat exchangers may also contribute to levels of these metals in the effluent (Wilkie, 2000).

(B) Since the petitioned substance is not requested for use in a pesticide, it is not, by definition, an inert. The previous paragraph provides sufficient information to determine eligibility of the substance under OFPA; however, the inert status of the substance is briefly described. The U.S. EPA has not classified the petitioned substance (vinasse, stillage, etc.) as an inert in Lists 1–4; however, phenol is included in List 1, inert ingredients of toxicological concern, and has been observed as a minor organic component of some vinasse samples (see Table 1). Phenolic compounds in stillage are derived from lignin (i.e., tannic and humic substances) naturally present in the feedstock or from industrial equipment contaminated with the chemicals due to unrelated processing operations (Wilkie, 2000).

<u>Evaluation Question #2:</u> Describe the most prevalent processes used to manufacture or formulate the petitioned substance. Further, describe any chemical change that may occur during manufacture or formulation of the petitioned substance when this substance is extracted from naturally occurring plant, animal, or mineral sources (7 U.S.C. § 6502 (21)).

Vinasse is a byproduct in the production of ethanol from the residues remaining after crystallization of sucrose from beet or cane juice. Bagasse, molasses, and filtercake are examples of other products resulting from this industrial process. Below are brief descriptions of sugarcane processing steps that ultimately lead to the production of vinasse used for various agriculture purpose outlined in "uses of the substance."

Cane Juice Production

Vinasse production begins with the growing and harvesting of sugarcane. The sugarcane is then usually milled across a tandem mill at a sugarcane factory to extract the cane juice. The crushed cane fibers exiting the last mill are referred to as bagasse. Following extraction, the cane juice is filtered to remove large particles and clarified to remove turbid and suspended particles using heat and lime (i.e., calcium oxide or calcium hydroxide) and polyanionic synthetic polyacrylamide flocculants. Clarification results in the formation of an insoluble particulate mass, which is separated from the limed juice by gravity and discarded or recycled back into the juice to maximize sugar yields (U.S. EPA, 1997).

Sucrose Crystallization and Processing

In order to facilitate the crystallization process, cane juice is concentrated to cane syrup. The clarified juice is initially passed through heat exchangers to preheat the juice and then proceeds to multiple-effect evaporator stations. Heating continues through a series of typically five evaporators; as the temperature decreases from an earlier evaporator to the next, the pressure inside each evaporator likewise is decreased to facilitate evaporation at lower temperatures. The final syrup is generally 65% solids and 35% water. In some factories (not common in the U.S.), the syrup can then be clarified using lime, phosphoric acid and polymer flocculants analogous to those used in water treatment (e.g., synthetic flocculants based on polyacrylamide), aerated, and filtered. Sucrose crystallization begins in vacuum pans, where the syrup is boiled until it reaches a supersaturated stage. At this stage, "seeding" or "shocking" the supersaturated solution initiates crystallization. Once the capacity of the vacuum pan is reached and the final mixture of liquor and crystals (i.e., massecuite) is formed, the mixture is discharged to the crystallizer, which maximizes sugar crystallization from the liquor. Centrifugation of the massecuite separates sugar crystals from the mother liquor (i.e., molasses). The crystals are washed with water and the wash water centrifuged from the crystals (U.S. EPA, 1997).

The mother liquor (i.e., molasses) and wash water from the first crystallization are returned to the vacuum pan, reboiled to form a second massecuite, which in turn yields a second batch of sucrose crystals. A third iteration of the entire process provides low grade sugar, typically mingled with syrup or used as a "seeding" solution in vacuum pans, and a final molasses. The final batch of molasses, known as blackstrap molasses, is a heavy, viscous material primarily used as a cattle feed supplement. Blackstrap molasses is also used in the production of ethanol, yeast, and organic acids, leaving vinasse as the primary byproduct (U.S. EPA, 1997). The raw sugar is then sent to a refinery where it is melted into a syrup and similar unit processes occur (e.g., clarification, evaporation, and crystallization) to produce refined sugar and a higher quality, food grade molasses.

Sugarbeet processing

Sugarbeets are processed directly into refined sugar in one factory following a similar manner to sugarcane but with some differences. Long, thin strips of cut sugarbeet are conveyed to continuous diffusers, in which sucrose is extracted through the application of hot water (50–80 ºC). Sulfur dioxide, chlorine, ammonium bisulfite or commercial FDA-approved, food-grade biocides are sometimes used as disinfectants. Raw juice, the sugar-enriched water that flows from the outlet of the diffuser, contains 10–15% sugar and undergoes clarification following the extraction procedure. Clarification begins with filtration of the raw juice to remove any small particles that may inhibit crystallization. The mixture is then heated (80–85 ºC) and proceeds through the carbonation process. Milk of lime (a slurry of calcium hydroxide) is added to the mixture in the first carbonation tank to adsorb impurities in the mixture. Carbon dioxide (CO_2) gas is then bubbled through the mixture to precipitate the lime as insoluble calcium carbonate crystals that settle out in the clarifier. At this point, the juice is again carbonated with CO_2 to remove the remaining lime and impurities. The calcium carbonate crystals are filtered from the juice and a small amount of sulfur dioxide (SO_2) is added to acidify the solution and inhibit any reactions leading to darkening of the juice. Following these initial processing steps, the juice is subjected to evaporation and crystallization procedures similar to those described above for cane juice (U.S. EPA, 1997).

Molasses Fermentation

There are a number of industrial processes that utilize molasses as a fermentation medium, all producing vinasse as a byproduct.

Ethanol production, and specifically rum-making, involves the fermentation of musts made of raw materials (i.e., cane juice, molasses) diluted with water followed by distillation of the fermented media. As a byproduct of cane sugar manufacturing, molasses has been the primary source of fermentable sugars for the rum industry since the 16th century. Yeast strains of the genus *Saccharomyces*, *Schizosaccharomyces*, *Pichia*, *Hansenula*, *Candida*, and *Toulopsis* are traditionally used to perform the alcoholic fermentation of diluted molasses (Fahrasmane, 1998). Saccharomyces cerevisiae, for example, has provided ethanol yields of 53 g L^{-1} in a medium containing 250 g L^{-1} total reducing sugars (Roukas, 1996). Recently, methods utilizing the bacterial strain *Zymomonas mobilis* have been developed for ethanol production, achieving yields of 55.8 g L^{-1} at a lower sugar concentration of 200 g L^{-1} (Cazetta, 2007).

Molasses is generally less contaminated with bacterial flora than cane juice, as a large portion of the non-sporulated bacteria is destroyed during sugar production. Notwithstanding, must components are frequently subjected to bacteriostatic or sterilizing thermal (steam) treatments to control any bacterial flora that may otherwise excrete undesired organic compounds into the fermentation medium (Fahrasmane, 1998). The molasses-based fermentation medium may also be treated with small quantities (~0.3 mg/L) of antibiotics, such as penicillin (Borzani, 1957) and tetracycline (Aquarone, 1960). However, the extent of this practice in current rum-making operations is uncertain. If added, it is possible that these antibiotics will not be fully degraded during the fermentation and ethanol distillation processes; a certain amount could remain in vinasse derived from antibiotic-treated fermentation mediums. Bacteriosides such as chlorine dioxide (Sumner 2011), ammonium bifluoride or quaternary ammonium compounds may also be used to control bacterial contamination (Murtagh, 1999). With the exception of chlorine dioxide, residues of these compounds may persist in vinasse. Finally, acidification of the media to a lower pH (i.e., pH = 4–5) using sulfuric acid generally precedes the fermentation step as a protective measure (Fahrasmane, 1998).

Fermentation procedures utilizing molasses have also been developed for the synthesis of amino acids, organic acids, and flavoring agents. Lactic acid, an important feedstock in the chemical industry, can be produced through the bacterial or fungal fermentation of molasses. Optimized conditions using the bacterial strains *Lactobacillus delbrueckii* and *Enterococcus faecalis* in combination with a molasses-based medium afforded lactic acid at concentrations of 90.0–95.7 g L^{-1} (Wee, 2006). Lactic acid yields as high as

166 g L^{-1} were reported for the fermentation of molasses using the *Lactobacillus delbrueckii* mutant Uc-3, generated via ultraviolet mutagenesis (Dumbrepatil, 2008). As a final example, three strains of the yeast *Williopsis saturnus* were employed for the fermentative production of isoamyl acetate, a natural banana flavor compound, using sugar beet molasses as the carbon source (Yilmaztekin, 2008).

Distillation

Distillations are commonly used to separate the chemical of interest (e.g., ethanol, lactic acid) from other components of the fermentation mixture. Details for the distillation of heavy and light rum are given below as examples of distillation procedures that leave vinasse as a residue.

Batch distillations are generally used for the production of heavy rums, while light rums require continuous distillation processes. In its simplest form, batch distillation involves the heating of the fermented molasses solution such that alcohol and other volatile compounds are distilled out of the mother liquor. Once vaporized, these volatile components proceed through the vapor pipe, condense in a cooled metal coil (i.e., condenser coil), and subsequently flow to the storage tank. The first fraction distilled may contain more of the volatile, pungent chemicals and is typically discarded. Distillation is continued until most of the alcohol has been distilled out of the fermented molasses material. Following the first distillation, the residue (i.e., vinasse or stillage) is removed from the pot and the distillate is returned from the storage tank to the pot to be redistilled following the above procedures. The term "vinasse" or "stillage" refers to the combination of all pot residues recovered during the distillation process (Martagh, 1999).

Vinasse Processing

The generated vinasse may be used in its diluted form, concentrated, or further processed for various agricultural applications (Parnaudeau, 2008). Please refer to Figure 1 below for a brief summary of the conversion of molasses to diluted and concentrated vinasse. Processes for fractioning vinasse to obtain separate organic and inorganic fractions have also been developed (Paananen, 2000). In this process, sulfuric acid is added to free the potassium contained within vinasse in the form of its sulfate salt. This procedure ultimately generates two fractions: (1) an organic fraction comprised of organic acids for use as a livestock feed additive and (2) an inorganic fraction comprised primarily of potassium salts for use as a fertilizer (Paananen, 2000). Methods for the combined distillation of ethanol and concentration of vinasse have also appeared in the recent patent literature (Almeida, 2010).

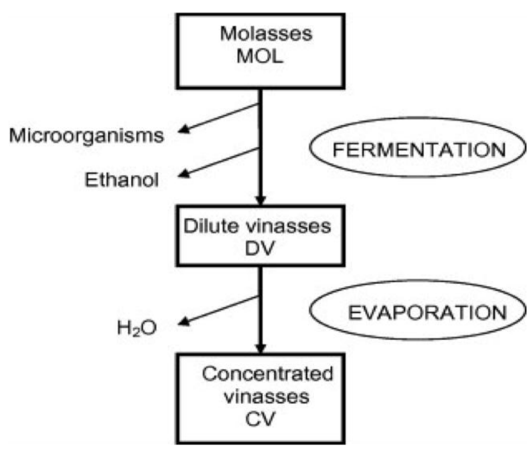

Figure 1. Industrial fermentation process leading to vinasse generation (Parnaudeau, 2008)

<u>Evaluation Question #3:</u> Discuss whether the petitioned substance is formulated or manufactured by a chemical process, or created by naturally occurring biological processes (7 U.S.C. § 6502 (21)).

Fermentation followed by distillation is the most common naturally occurring biological process used to generate vinasse from cane and beet molasses. Yeast strains of the genus *Saccharomyces*, *Schizosaccharomyces*, *Pichia*, *Hansenula*, *Candida*, and *Toulopsis* are traditionally used to perform the alcoholic fermentation of diluted molasses (Fahrasmane, 1998). Recently, methods utilizing the bacterial strain *Zymomonas mobilis* have been developed for the fermentative production of ethanol, achieving yields of

55.8 g L^{-1} (Cazetta, 2007). Optimized conditions using the bacterial strains *Lactobacillus delbrueckii* and *Enterococcus faecalis* in combination with a molasses-based medium afforded lactic acid at concentrations of 90.0–95.7 g L^{-1} (Wee, 2006). Other yeast and bacterial strains, including genetically modified microorganisms, are commonly used depending on the organic compound of interest.

Sulfur dioxide, chlorine, ammonium bisulfite or commercial FDA-approved biocides are sometimes used to disinfect cane juice. Milk of lime (a slurry of calcium hydroxide) is added to cane and beet juice during the clarification step; however, this material is mostly removed by filtration during clarification. The pH of the fermentation broth may be lowered (pH = 4–5) using sulfuric acid to minimize the risk of bacterial contamination. In addition, antibiotics (e.g., penicillin) and bactericides (e.g., chlorine dioxide) are potentially added to control bacteria present in the molasses feedstock. Due to the inclusion of synthetic substances (i.e., sulfur dioxide and sulfuric acid) in the overall production of vinasse, the Organic Materials Research Institute (OMRI) removed vinasse from the OMRI List of Approved Products as an organic fertilizer (OMRI, 2013).

A synthetic substance is defined by the NOP as being "formulated or manufactured by a chemical process or by a process that chemically changes a substance extracted from naturally occurring plant, animal, or mineral sources, except that such term shall not apply to substances created by naturally occurring biological processes." According to this definition and the classification of fermentation as a naturally occurring biological process, vinasse would constitute a nonsynthetic (natural) substance. However, the potential use of genetically engineered microorganisms and chemical substances not allowed on the National List during the fermentation of molasses should be weighed in determining the status of vinasse as nonsynthetic (natural) or synthetic.

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